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(71) Applicant *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MARTINEZ, Alberto [GB/GB]; Zeneca Agrochemicals, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY (GB). JEPSON, Ian [GB/GB]; Zeneca Agrochemicals, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6ET (GB). FRAY, Rupert, George [GB/GB]; University of Nottingham, Faculty of Science, School of Biological Science Plant Science Division, Sutton Bonnington Campus, Loughborough, Leicester LE12 5RD (GB).

(74) Agents: KENT, Lindsey, Ruth et al.; Zeneca Agrochemicals, Intellectual Property Department, Jealott's Hill Research Station, P.O. Box 3538, Bracknell, Berkshire RG42 6YA (GB).

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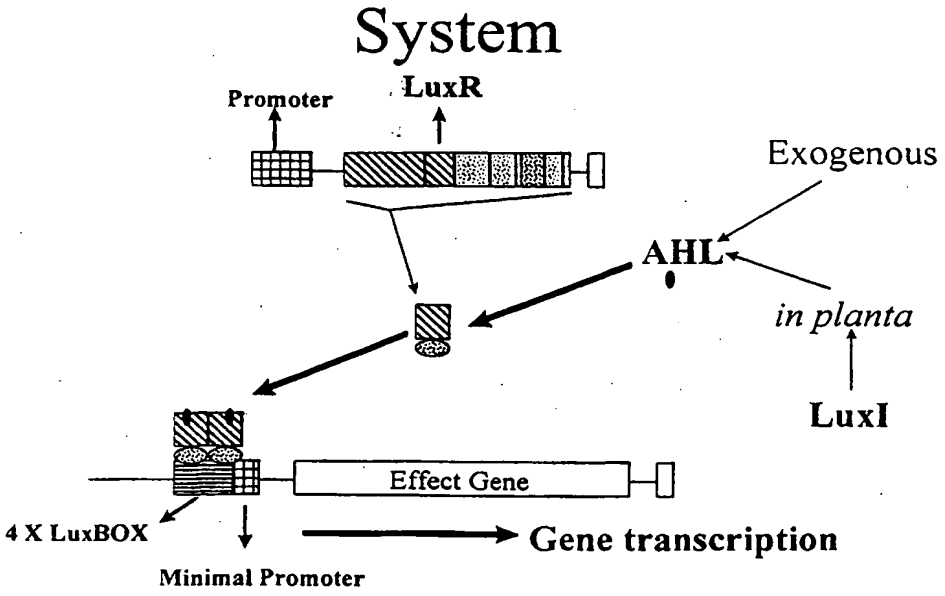

(57) Abstract

The present invention relates *inter alia*, to a method of initiating transcription of a target gene in a eukaryotic cell comprising: (a) providing a eukaryotic cell which is capable of producing a response protein; and (b) inserting into the genome of said cell a polynucleotide defining an inducible promoter sequence operably linked to and capable when induced of initiating transcription of said target gene; and (c) applying to said cell a chemical inducer capable of binding to said response protein whereby said chemical inducer binds to said response protein to form an inducing complex which binds to and induces said inducible promoter thereby initiating transcription of said target gene.

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GENE SWITCH

The present invention relates *inter alia*, to the induction of gene expression in a eukaryote by the application of a chemical inducer to the eukaryote and to materials and methods for achieving induction. Such systems are referred to as "gene switches".

In particular, the present invention relates to a method of controlling expression of a target gene in a plant, animal or yeast.

Bacterial cells have the ability to respond to the surrounding environment. The response to different environmental cues is essential for survival of bacteria. It is apparent that individual bacteria in a population are also able to sense the density and state of the local bacterial population (the "quorum") of which they are members. That is, an individual bacterium can detect the presence of like bacteria in the surrounding environment. Quorum sensing allows bacteria to synchronise growth, development which when a minimal population level is reached initiates a concerted response from the population.

In the case of *Photobacterium fischeri*, *N*-(3-oxohexanoyl)-L-homoserine lactone or autoinducer regulates bioluminescence in a cell density-dependent manner. There are two main genes in the *lux* operon of *P. fischeri* involved in the signal production and signal detection. *LuxI* is the gene involved in the biosynthesis of the homoserine lactone but the mechanism by which this takes place is unclear. It has been proposed that *S*-adenosylmethionine and coenzymeA or the acyl carrier protein adduct of 3-oxohexanoic acid are substrates for the *luxI* gene in *P. fischeri*.

In bacteria, the autoinducer regulates expression of the *luxI* gene and thus creates a positive autoregulation of autoinducer synthesis. *LuxR*, the response regulator or autoinducer receptor, is a protein involved in responding to the presence of *N*-(3-oxo)hexanoyl-L-homoserine lactone (OHHL) in *P. fischeri*. At the C-terminal end *LuxR* contains a DNA binding domain and a transcriptional activator. *LuxR* C-terminal end shows amino acid homology to transcriptional activators known as the two component environmental-sensing systems such as UhpA, FixJ and NarL. *LuxR* is thought to interact with DNA as a homodimer to a palindrome within the *luxI* operator sequence termed *lux* box. The N-terminal end of the protein is called the

receptor module as it has no similarity to the two-component environmental sensing systems.

Quorum sensing systems are found in other bacteria and may be activated by different homoserine lactones. Such is the case in *P. aeruginosa* PAO1, *LasI* directs the synthesis of the autoinducer *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), which activates the positive transcriptional activator, LasR (Winson et al., 1995). Moreover, in the same *P.auruginosa*, PAO1, a second signalling pathway termed *vsm* containing *vsmR* and *vsmI* genes was isolated. The *vsmI* gene product is involved in production of *N*-butanoyl-L-homoserine lactone (BHL) and *N*-hexanoyl-L-homoserine lactone (HHL). These compounds are present in the spent culture supernatants of *P. aeruginosa* and when either BHL or HHL to PAN067, a pleiotropic *P. aeruginosa* mutant unable to synthesize either of these autoinducers, restored elastase, chitinase, and cyanide production (Winson *et al.*, 1995). Other evidence suggesting the presence of different homoserine inducers in one species has recently been observed in *Vibrio anguillarum* (Milton *et al.*, 1997) and *P. aeroginosa* (Pesci *et al.*, 1997). Furthermore, Table 1, shows examples of characterised systems where different compounds are known to be inducers of different receptor molecules in different bacteria.

Table 1.

Organism	**Signal generator**	**Response Regulator**	**Signal Molecule**	**GenBank Accession number**	**References**
Aeromonas hydrophila	AhyL	AhyR	unknown	X89469	
Agrobacterium tumefaciens	TraI	TraR	*N*-(3-oxo)-octanoyl-L-homoserine Lactone (OOHL)	L17024, L22207	Fuqua and Winans, 1994; Hwang *et al.*, 1995
Chromobacterium violaceum	CviI	CviR	*N*-hexanoyl-L-homoserine lactone (OHL)		Winson, *et al.*, (1994)
Enterobacter agglomerans	EagI	unknown	*N*-(-3-oxo)hexanoyl-L-homoserine lactone (OHHL)	x74300	Swift *et al.*, 1993
Erwinia carotovora sudsp carotova	CarI	CarR	OHHL	U17224, X72891, X74299, X80475	McGowan *et al* .,1995
Erwinia stewartii	EsaI	EsaR	OHHL	L32183, L32184	Beck von Bodman and Farrand, 1995

Escherichia coli	unknown	SdiA	unknown	Xo3691	Sitnikov *et al* 1995
Photobacterium fischeri	LuxI	LuxR	OHHL,OOHL	M19039, M96844, M25752	Meignhen, 1994; Devine *et al*, 1988
Pseudomonas aeruginosa	LasI	LasR	*N*-(-3-oxo)-dodecanoyl-L-homoserine lactone (OdDHL)	M59425	Winson *et al* 1995; Jones *et al*, 1993; Pearson *et al.*, 1995.
	VsmI	vsmR	*N*-butanoyl-L-homoserine lactone (BHL), HHL	L08962, U11811, U15644	Winson *et al.*, 1995; Williams *et al*, 1996 Latifi *et al* 1995; Ochsner and Reiser, 1995.
Pseudomonas aureofaciens	PhzI	PhzR	unknown	L32729, L33724	Wood and Piersen, 1996
Rhizobium leguminosarum	unknown	RhiR	*N*(-3-hydroxy)-tetradecanoul-L-homoserine lactone (HtDeHL)	M98835	Fuqua *et al.*, 1994; Gray *et al.*, 1996.
Serratia liquefaciens	SwrI	unknown	BHL	U2823	

Aeromonas hydrophila	ahyI	ahyR	BHL		Swift et al., 1997
Aeromonas salmonicida	AsaI	unknown	BHL, *N*-hexanoyl-L-homoserine lactone		Swift *et al.*, 1997
Vibiro anguillarum	vanI	vanR	*N*-(3-oxo-decanoyl)-L-homoserine lactone (ODHL)		Milton *et al.*, 1997
Vibrio harveyi	LuxLM	LuxN	*N*-(3-hydroxy)-butanoyl-L-homoserine lactone (HBHL)	L13940	Meighen, 1994; Bassler *et al.*, 1994.
Yersinia enterocolitica	YenI	YenR	OHHL,HHL	X76082	Throup *et al.*, 1996.

The present invention therefore seeks to provide *inter alia*, methods and materials for the induction of gene expression in eukaryotes by the application of chemical inducers to the eukaryote.

According to the present invention there is provided a method of initiating transcription of a target gene in a eukaryotic cell comprising:

(a) providing a eukaryotic cell which is capable of producing a response protein;

(b) inserting into the genome of said cell a polynucleotide defining an inducible promoter sequence operably linked to and capable when induced of initiating transcription of said target gene; and

(c) applying to said cell a chemical inducer capable of binding to said response protein whereby said chemical inducer binds to said response protein to form an inducing complex which binds to and induces said inducible promoter thereby initiating transcription of said target gene.

The eukaryotic cell may already contain the mechanisms to produce the said response protein or may alternatively be provided with them by inserting into the said cell, a polynucleotide which provides for the production of the response protein using techniques well known within the art. The inducible promoter for use in the above mentioned method may comprise the nucleotide sequence depicted as SEQ ID No. 2 and the response protein may comprise the amino acid sequence depicted in SEQ ID No. 16. Alternatively the inducible promoter may comprise a functional variant such as a polynucleotide which is the complement of one which binds to SEQ ID No. 2 at a temperature of between 60°C and 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS wherein the said polynucleotide is still capable of acting as an inducible promoter upon binding with the said inducing complex. In particular the response protein for use in the method of the present invention may be encoded by the polynucleotide comprises the sequence depicted in SEQ ID No. 5. Alternatively the polynucleotide encoding the said response protein may comprise the complement of one which binds to SEQ ID No. 5 at a temperature of between 60°C and 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline

containing 0.1% SDS wherein the said polynucleotide still encodes a protein which is capable of forming an inducing complex with the said chemical inducer. It is particularly preferred that the polynucleotide defining the inducible promoter according to the present invention contains the region depicted as SEQ ID No. 21.

The method of present invention is particularly applicable to initiating transcription in cells of plants, more particularly in the cells of: melons, mangoes, soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, peaches, grape, strawberries, carrot, lettuce, cabbage, onion, citrus or nut plants. In particular the method of the present invention may be used to initiate transcription in a variety of tissues, including roots, leaves, stems and reproductive tissues. The chemical inducer which may be used in the above mentioned method is N-(3-oxo)hexanoyl-L-homoserine lactone or a functional equivalent thereof and may be applied to the plant cell as part of an agriculturally acceptable formulation. Alternatively the chemical inducer may be produced within the plant by inserting into the genome of the plant a polynucleotide encoding a protein which provides for the production of the chemical inducer within the plant. This polynucleotide may, for example, be under the transcriptional control of a constitutive promoter, a gene switch (such as the alcA/R, *Heliothis* ecdysone and GST-27 gene switch), wound inducible, tissue or temporal promoters. The advantage of producing the chemical inducer *in planta* is that there is no need to spray or exogenously treat plants to induce gene expression.

Further alternative inducible promoters, response proteins and chemical inducers which may be used in the method of the present invention are referenced in Table 1. For example, the promoter sequence or a part thereof may obtainable from the vanI gene of *Vibiro anguillarum* and the response protein encoded by the vanR gene may be used with the chemical N-(3-oxo-decanoyl)-L-homoserine lactone (ODHL) or a functional variant thereof.

The polynucleotide encoding the said response protein in the method referred to above may be bounded by a promoter and a terminator sequence and in particular the promoter may be inducible such as the Alc A/ R switch system, the GST switch system and the ecdysone switch. Alternatively the promoter may be constitutive such

as cauliflower mosaic virus 35S/19S, maize Ubiquitin and *Arabidopsis* Ubiquitin 3 or may be developmentally regulated specific promoter such as those controlling expression of gene required during seed formation, germination such as cysteine proteinases (as specified in our International Publication No WO 97/35983) and malate synthase.

The target gene in the method referred to above may be any gene of interest, for example, β-glucuronidase; *Bacillus thuringenesis* toxin; barnase or barstar.

In a further aspect of the present invention there is provided a method of providing plants containing an inducible target gene comprising:

(a) inserting into a plant cell which cell provides for production of a response protein a polynucleotide defining an inducible promoter sequence operably liked to said target gene; and (b) regenerating morphologically normal fertile plants thereof; and (c) applying to the population of regenerants a chemical inducer or a functional variant thereof capable of binding to said response protein, whereby said chemical inducer binds to said response protein to form an inducing complex which binds to and induces said inducible promoter thereby initiating transcription of said target gene; and (d) selecting those plants which are expressing the said target gene. The inducible promoter sequence referred to in the preceding paragraph may comprise the nucleotide sequence depicted as SEQ ID No. 2 or a functional variant thereof and the response protein comprises the amino acid sequence depicted as SEQ ID No 16 or a functional variant thereof and the said chemical inducer is N-(3-oxo)hexanoyl-L-homoserine lactone or a functional equivalent thereof. In particular the inducible promoter sequence may contain the nucleotide sequence depicted as SEQ ID No. 10 or a functional variant thereof and the response protein may comprises the amino acid sequence depicted as SEQ ID No 17 or a functional variant thereof and the said chemical inducer may be N-(3-oxo)dodecanoyl-L-homoserine lactone or a functional equivalent thereof. Alternatively, the said inducible promoter sequence may contain the nucleotide sequence depicted as SEQ ID No. 12 or a functional variant thereof and the response protein may comprise the amino acid sequence depicted as SEQ ID No 18 or a functional variant thereof and the said chemical inducer may be N-(3-oxo)octanoyl-L-homoserine lactone or a functional equivalent thereof.

The present invention also provides plants produced according to the method of the preceding paragraph which plants may be selected from the group consisting of: melons, mangoes, soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, peaches, grape, strawberries, carrot, lettuce, cabbage, onion, citrus or nut plants.

The method employed for transformation of plant cells is not especially germane to the present invention and any method suitable for the target plant may be employed. For example, transgenic plants are obtained by regeneration from the transformed cells. Numerous transformation procedures are known from the literature including agroinfection using *Agrobacterium tumefaciens* or its Ti plasmid, electroporation, microinjection or plants cells and protoplasts, microprojectile transformation. All of these methods are well known within the art. The present invention may also be applied to any plant for which transformation techniques are, or become, available.

In a further aspect of the present invention there is provided a DNA construct which comprises a first polynucleotide region which comprises an inducible promoter sequence operably liked to and capable when induced of initiating transcription of a target gene and a second polynucleotide region which provides for the production of a response protein wherein upon contact of the said response protein with a chemical inducer the said response protein binds the chemical inducer to produce an inducer complex which binds the said inducible promoter sequence thereby initiating transcription of the said target gene. The said first polynucleotide of the DNA construct may comprise the nucleotide sequence depicted as SEQ ID No. 2 or a functional variant thereof and the second polynucleotide region may comprise a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 16 or a functional variant thereof. Alternatively the said first polynucleotide may comprise the nucleotide sequence depicted as SEQ ID No. 10 or a functional variant thereof and the second polynucleotide region may comprise a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 17 or a functional variant thereof. Alternatively, the said first polynucleotide may comprise the nucleotide sequence

depicted as SEQ ID No. 12 or a functional variant thereof and the second polynucleotide region may comprise a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 18 or a functional variant thereof. The said second polynucleotide region referred to above may comprise a promoter operably linked to the polynucleotide encoding the said response protein. The said promoter may be inducible and constitutive or developmentally controlled.

The present invention still further provides the use of a DNA construct referred to above in the production of a plant containing a target gene of which the expression may be controlled by the application of a chemical inducer to the said plant.

In a further aspect of the present invention there is provided a method of screening compounds in a bioassay comprising applying an amount of a chemical to a plant which plant contains a polynucleotide comprising an inducible promoter operably linked to a region encoding a reporter protein and which plant is also capable of producing a response protein and testing the said plant for the production of the said reporter protein. The chemical could be applied to the plant and the activity of the reporter gene monitored for e.g. improved activity, mobility or stability or to assess if the chemical had an inhibitory effect on the response protein which resulted in a decrease in activity of the reporter gene.

In a further aspect of the present invention there is provided a method of selectively controlling pests in a field which field comprises crop plants and pests wherein the plants are those obtained according to the methods referred to above and the said target gene encodes a target protein which is capable of controlling the said pests said method comprising applying to the plants an amount of a chemical inducer which is sufficient to bind to the said response protein to produce the said inducing complex which is capable of initiating transcription of the target gene which provides for the production of the target protein in an amount which is sufficient to control the said pests.

In a further aspect of the present invention there is provided a method of providing a plant which contains a target gene which is inducibly controlled comprising:

(a) inserting into a first plant cell a polynucleotide comprising a first inducible promoter operably linked to a target gene and regenerating a first morphologically normal fertile plant therefrom;

(b) inserting into a second plant cell a polynucleotide comprising a promoter operably linked to a region encoding a response protein which is capable of binding to a chemical inducer to produce an inducing complex which is then capable of binding the said inducible promoter to allow for the initiation of transcription of the said target gene and regenerating a second morphologically normal fertile plant therefrom;

(c) cross pollinating said first plant with the said second plant or said second plant with said first plant and harvesting the seed therefrom;

(d) growing said seed and applying to the resultant plants an amount of said chemical inducer which provides an inducing complex capable of binding the said inducible promoter to allow for the initiation of transcription of the said target gene.

The term "functional variant" with respect to a polynucleotide encoding the inducible promoter of the present invention includes variant sequences which are the complement of a sequence which hybridises to the inducible promoter sequence at a temperature of between 60°C and 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS and which are still capable of acting as an inducible promoter.

The term "functional variant" with respect to the response protein includes variant proteins obtained by conservative substitutions within the amino acid sequence which substitutions do not significantly adversely affect the ability of the response protein to bind the chemical inducer. In particular substitutions may be made between the following amino acid groups *viz.*

(a) Alanine, Serine, Glycine and Threonine

(b) Glutamic acid and Aspartic acid

(c) Arginine and Lysine

(d) Isoleucine, Leucine, Valine and Methionine

(e) Phenylalanine, Tyrosine and Tryptophan

The term "transgenic" in relation to the present invention does not include a wild type regulator promoter in its natural environment in combination with its associated functional gene in its natural environment.

The term "target gene" with reference to the present invention means any gene of interest. A target gene can be any gene that is either foreign or natural to the eukaryote in question.

The term "construct" - which is synonymous with terms such as "cassette", "hybrid" and "conjugate" - includes a target gene directly or indirectly attached to the regulator promoter, such as to form a cassette. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence intermediate the promoter and the target gene. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. Such constructs also include plasmids and phage which are suitable for transforming a cell of interest.

The term "expression system" means that the system defined above can be expressed in an appropriate organism, tissue, cell or medium. The system may comprise one or more constructs and may also comprise additional components that ensure to increase expression of the target gene by use of the regulator promoter.

One possible use of the inducible promoters of the present invention is in the control of male sterility. The anther is the site of male reproductive processes in flowering plants. It is composed of several tissues and cell types and is responsible for producing pollen grains that contain the sperm cells. The tapetum is a specialised tissue which plays a critical role in pollen formation. It surrounds the pollen sac early in pollen development, degenerates during the latter stages of development and is not present in an organised form in the mature anther. The tapetum produces a number of compounds which aid pollen development or are incorporated into the pollen outer wall and it has been demonstrated that many of the natural male sterility mutations have impaired tapetum differentiation or function.

Tapetal tissue is therefore critical to the formation of functional pollen grains. A number of genes have been identified and cloned that are specifically expressed in tapetal tissue. They include Osg6B, Osg4B (Tsuchiya *et al.* 1994, Yokoi, S *et al.* 1997), pE1, p T72 (WO9213957), p CA55 corn (WO92/13956) , TA29, TA13,(Seurinck *et al.* 1990), RST2 corn (WO9713401), MS14,18,10 and A6, A9

from *Brassica napus* (Hird *et al.* 1993). Anther specific clones have been isolated from a number of species Bp4A and C (Albani *et al.* 1990), chs petunia (Koes *et al.* 1989), rice (Xu *et al.* 1993, Zou *et al.* 1994), amongst others. In higher plants the female reproductive organ is represented by the pistil, composed of the ovary, style and stigma. The gynoecium has been shown to contain up to 10,000 different mRNAs not present in other organs (Kamalay and Goldberg 1980). These include regulatory genes responsible for controlling pistil development as well as "downstream" ones encoding proteins associated with differentiated cell types in the pistil. Genes governing self-incompatibility and their homologues are one class of gene with pistil predominant expression patterns (Nasrallah *et al.* 1993). Other cloned genes which are applicable as target genes in the present invention include β glucanase (Ori *et al.* 1990), pectate lyase (Budelier *et al.* 1990) and chitinase (Lotan *et al.* 1989) which are expressed in the transmitting tissue and a proteinase inhibitor (Atkinson *et al.* 1993) which are expressed in the style. Others are pathogenesis related or are homologues of genes involved in the cleavage of glycosidic bonds. These enzymes may facilitate pollen tube growth by digesting proteins in the tissue through which the pollen tube grows. A number of female sterile mutants have been identified in *Arabidopsis*. For example, sin1 (short integument) (Robinson-Beers *et al.* 1992) and bel1 (bell) (Robinson-Beers *et al.* 1992) affect ovule development. In the short integument a mutation blocks megasporogenesis at the tetrad stage (Elliot,R.C, *et al.* 1996, Klucher,K.M, 1996). A lethal ovule 2 mutation has been observed but not cloned in maize (Nelson *et al.* 1952). Pistil specific basic endochitinases have been cloned from a number of species (Ficker *et al.* 1997, Dzelzkalns *et al.* 1993, Harikrishna *et al.* 1996, Wemmer *et al.* 1994) and extensin-like genes have been shown to be expressed in the styles of *Nicotiana alata* (Chen C-G, *et al.* 1992). The following are ovule specific clones ZmOV23,13, (Greco R., *et al.* unpublished), OsOsMAB3A (Kang H.G., *et al.* 1995), ZmZmM2 (Theissen G., *et al.* 1995) and stigma specific stig1 (Goldman, M.H *et al.* 1994) , STG08, STG4B12 (EP-412006-A). Mariani *et al.* used the promoter from the STIG1 gene to drive expression of barnase in the stigmatic secretory zone.

In summary then, the present invention therefore provides a gene switch which is operably linked to a foreign gene or a series of foreign genes whereby expression of

said foreign gene or said series of foreign genes may be controlled by application of an effective exogenous inducer. The gene switch of the present invention, therefore, when linked to an exogenous or foreign gene and introduced into a eukaryote by transformation, provides a means for the external regulation of expression of that foreign gene.

It is possible to use one, two or more of these inducible promoter regions according to the present invention to activate different processes in plant cells, thereby obtaining a plant which will have multiple inducible cassettes all controlled by for example, different homoserine lactones. Also, a plant may contain, for example, an inducible promoter according to the present invention in conjunction with other switch type mechanisms examples of which include inducible promoters include the Alc A/ R switch system described in International Publication No. WO. 93/21334, the GST switch system described in International Publication Nos WO 90/08826 and WO 93/031294 and the ecdysone switch described in our International Publication No. WO 96/37609.

The methods and products of the present invention may also be used to control expression of foreign proteins in eukaryotes such as yeast and mammalian cells. Many heterologous proteins for different applications may be produced by expression in such eukaryotic cells. The present invention is advantageous in that it provides control over the expression of foreign genes in such cells. It also provides a further advantage, particularly in yeast and mammalian cells, where accumulation of large quantities of a heterologous protein can damage the cells, or where the heterologous protein is damaging such that expression for short periods of time is required in order to maintain the viability of the cells. The inducible system of the present invention also has applicability in gene therapy as it allows the timing of the therapeutic gene to be controlled. The present invention is therefore not only advantageous in transformed mammalian cells but also to mammals *per se*. Furthermore, the present invention may be used to switch on genes which produce potentially damaging or lethal proteins. Such a system may be employed in the treatment of cancer in which cells are transformed with genes which express proteins which are lethal to the cancer. The timing of the action on such proteins on the cancer cells may be controlled using the switch of the present invention.

Various preferred features and embodiments of the present invention will now be described by way of non-limiting examples with reference to the accompanying Figures of which:-

Figure 1 shows a schematic representation of the general structure of the response regulator protein of the bacterial quorum sensing system.

Figure 2 shows a schematic representation of the homoserine lactone gene switch system.

Figure 3 is a plasmid map of the reporter gene construct, p221.9lux6.

Figure 4 is a plasmid map of p221.9lux2.

Figure 5 Plasmid map of p221.9lux3.

Figure 6 Plasmid map of p221.9luxC.

Figure 7 Plasmid map of p221.9Lpro.

Figure 8 Depicts the expression vector containing the LuxR gene in the pDH51LuxR plasmid.

Figure 9 Shows the expression cassette containing the enhanced N-terminal fusion protein of SV40-NLS-Gal4-LuxR in the plasmid pDH51SVLuxR.

Figure 10 Shows the expression cassette containing the enhanced C-terminal fusion of LuxR-SV40-NLS-Gal4 in the plasmidDH51LuxRSV.

Figure11 is the plasmid map of p221.9lasbox. Containing one copy of the LasR Box.

Figure 12 is a plasmid map of the transient expression construct containing LasR, pSinLASR.

Figure 13 expression plasmid pFunLuxR for expression in monocotyledon protoplasts.

Figure 14 expression plasmid pFunLasR for expression in monocot protoplasts.

Figure 15 expression plasmid pFunTraR for expression in monocot protoplasts.

Figure 16 reporter plasmid p221.9trabox1 containing tra box sequence 1 described by Zhu and Winans, (1999)

Figure 17 reporter plasmid p221.9trabox2 containing tra box of sequence 2 described by Zhu and Winans, (1999).

Figure 18 is a plasmid map of binary vectors containing the effector and reporter cassettes, pAHL1.

Figure 19. is a plasmid map of binary vectors containing the effector and reporter cassettes, pAHL2.

Figure 20. is a plasmid map of binary vectors containing the effector and reporter cassettes, pAHL3.

Figure 21 is a plasmid map of binary vector containing *luxI* gene, pBDHELI.

Figure 22 is a plasmid map of pSB401 containing the Lux operon.

Figure 23 shows the root of a transgenic tobacco plant that was grown in tissue culture under aseptic conditions was placed on an LB agar plate and overlain with top agar containing *E. coli* with the plasmid pSB401 (Figure 22). This strain expresses the lux operon and will bioluminesce in response to OHHL and HHL. Bioluminescence in the region around the root was clearly seen with the naked eye.

Figure 24. Shows a leaf of a transgenic tobacco plant that was grown in tissue culture under aseptic conditions. The leaf was excised from the plant and placed on an LB agar plate and overlaid with top agar containing *Chromobacterium violaceum* indicator strain. The violet colour detected represent the induction of the indicator stain gene showing the extrusion of OHHL from the plan tissue into the bacterial medium.

Sequences

SEQ ID No. 1 is the *LuxI* box promoter region.

SEQ ID No. 2 is the *LuxI* promoter region.

SEQ ID No. 3 & 4 are the *LuxR*Bamh1 fragment.

SEQ ID No. 5 & 6 are the *LuxR* sequence/protein sequence.

SEQ ID No. 7 & 8 are the NVLuxR fusion flanked by BamHI and PstI sites/protein sequence.

SEQ ID No. 9 & 10 are the LuxRNV sequence/protein sequence.

SEQ ID No. 11 is a TraR1 fragment.

SEQ ID No. 12 is the TraR2 fragment.

SEQ ID No. 13 is the LasBox1 region.

SEQ ID No. 14 is the LasBox2 region.

SEQ ID No. 15, 16, 17 and 18 are the TraBox1,2,3,4 regions respectively.

SEQ ID No. 19 is the *LuxR* response protein sequence.

SEQ ID No. 20 is the *LasR* response protein sequence.

SEQ ID No. 21 is the *TrasR* response protein sequence.

SEQ ID No. 22 & 23 are the open reading frames of *LasR* and *TraR* respectively.

SEQ ID No.24 is the *Lux* Box promoter region.

Example 1. Transient expression of *LuxR* and reporter plasmid in Tobacco mesophyll protoplasts.

Preparation of Reporter gene expression cassette.

Six copies of the 20bp palindromic *lux* box sequence will be fused upstream of the -60CaMV minimal promoter which in turn is fused to the reporter gene GUS. The palindrome of sequence 5' GATCACCTGTACGATCGTACAGGT 3' (Sequence ID. 1) was self annealed and introduced into a *Bam*HI pBluescript vector. Sequence determination of a number of recombinants lead to the identification of a plasmid with 6 copies of the palindrome. The identified plasmid was digested with *Hin*dIII and *Sal*I to release the in 6 copies of the palindrome and introduced into a *Hin*dIII/*Sal*I p221.9 vector. p221.9 plasmid contains a -60CaMV minimal promoter fused to GUS downstream of the *Hin*dIII and *Sal*I unique cloning sites. A recombinant plasmid was identified and named p221.9lux6 (Figure 3). The same oligonucleotide was used to generate p221.9LuxR2 (Figure 4), p221.9LuxR3 (Figure 5) and p221.9LuxC (Figure 6). p221.9lpro was generated using the bacterial promoter containing the *Lux* box sequences (Figure 7 Sequence ID 2).

Preparation of *LuxR* expression vector.

The lux receptor was altered at both ends of the coding sequence. At the 5'end a plant Kozac consensus sequence was placed with an *Nco*I site at the ATG start of the coding sequence. Upstream from the Kozac consensus sequence a *Bam*HI unique site was introduced using PCR. The sense oligonucleotide was luxrbamh1 5' CCCGGATCCTAACAATGGGTATGAAAGACATAAATG 3' (Sequence ID.3)and the antisense primer was luxrbamh2 5' CGAACTCGAGTCATGATTTTAAAGTATGGGCAA-TCAATTG 3' (Sequence ID.4). The PCR reaction was carried out using *Taq* polymerase (2.5 U) in a reaction

buffer containing 100ng of template DNA, 100 ng of each oligonucleotide, 20 mM TRIS-HCl pH 8.4, 50 mM KCl, 10 mM $MgCl_2$, 50 mM dNTPs and using hot start conditions followed by 15 cycles of denaturing (94°C for 1 minute), annealing (66°C for 1 min) and synthesis (72°C for 3 min). The fragment was purified and digested using *Bam*HI/*Xho*I and was introduced into pDH51 *Bam*HI/*Sal*I vector to give pDH51luxR (*Xho*I and *Sal*I restriction enzymes produce compatible ends) (Figure 8). The sequence of the insert was determined and compared to the published *LuxR* sequence (Sequence ID. 5) (Devine et al., 1988).

Tobacco mesophyll transformation.

Tobacco shoot cultures cv. *Samsun*, were maintained on solidified MS medium + 3% sucrose in a controlled environment room (16 hour day / 8 hour night at 25°C, 55% R.H), were used as the source material for protoplasts. Leaves were sliced parallel to the mid-rib, discarding large veins and the slices were placed in CPW13M (13% mannitol, pH5.6, 860mmol/kg) for 1 hour to pre-plasmolyse the cells. This solution was replaced with enzyme mixture (0.2% cellulase R10, 0.05% macerozyme R10 in CPW9M (CPW13M but 9% mannitol), pH5.6, 600mmol/kg) and incubated in the dark at 25°C overnight (16 hours). The enzyme mixture was passed through a 75µm sieve and the filtrate was centrifuged at 600rpm for 3.5 minutes, discarding the supernatant. The pellet was resuspended in 0.6M sucrose solution and centrifuged at 600rpm for 10 minutes. The protoplasts were removed and diluted with CPW9M (pH5.6, 560mmol/kg) and pelleted by centrifuging at 600rpm for 3.5 minutes. The protoplasts were resuspended in CPW9M, counted, diluted to $2x10^6$/ml in MaMg medium (Negrutui *et al.*, 1987) and aliquoted at $4x10^5$ protoplasts per treatment. 20µg each of effector and reporter plasmid DNA (1mg/ml) were added followed by 200µl PEG solution (Negrutui *et al.*, 1987). The protoplasts were incubated at room temperature for 10 minutes before addition of 5ml MSP19M medium (MS medium, 3% sucrose, 9% mannitol, 2mg/l NAA, 0.5mg/l BAP, pH5.6, 700mmol/kg) in the presence or absence of ligand (N-(-3-oxohexanoyl)-L-homoserine lactone (OHHL)). The protoplasts were cultured in their tubes lying horizontally at 25°C and they were harvested for the GUS assay after 24 hours.

Transient Expression in *Nicotiana pumbaginifolia* suspension cells derived protoplasts

Protoplasts isolation

Protoplasts were isolated from *Nicotiana plumbaginifolia* suspension cells. Suspension cells were sub-cultured once a week in Np suspension medium in 250 ml Erlenmeyer flasks that were shaken at 100 RPM , at 25°C with 16h/8h light/dark regime. Protoplasts were isolated 2 or 3 days after subculture. Two or 2.5 g fresh weight of cells were incubated with 20 ml filter-sterilised enzyme solution and shaken at 40 rpm at 25°C. The enzyme solution comprised 1% cellulysin (Calbiochem 219466), 1 % Macerozyme RIO™ (Yakult, Tokyo), and 1% Driselase (Sigma™ D-9515) dissolved Artificial Sea Water Mannitol. After 3 h cell digestion in enzyme solution, they were washed through 100-, 50-um diameter sieves with W5 solution before being collected by centrifugation at 80 *x g* for 4 min. This method is also used for the isolation of wheat protoplasts.

Protoplasts transformation.

Protoplasts were resuspended in W5 medium at densities ranging from 0.1 to 0.2 x 10^6 /ml and sedimented for 4 min at 80 *g*. They were then taken up in 0.2 to 0. 5 ml of: 0.4 M mannitol, 15 mM $MgCl_2$, 0.1 % MES, 2 % glucose, pH 5.6 containing 15 µg plasmid DNA. Five minutes later poly-ethylene glycol (PEG) 4000 (Fluka™) at 40% (w/v) in 0.4 M mannitol and 0. 1 M $Ca(N0_3).4H_20$ pH 9.0 was added to give final PEG concentrations of 20%. After half an hour 1 ml 0.2 M $CaCl_2.2H_20$ were added, and the protoplasts centrifuged for 4 min at 40 *g*. The protoplasts were then cultured in transformation buffer at 0.1-0.2 x 10^6 protoplasts per ml during 48h at 25°C in the dark. This method is also applicable to the transformation of wheat protoplasts.

GUS Assays

Transient transformed tobacco protoplasts were harvested by centrifugation at 3000 rpm. by collecting them by centrifugation at 3000 rpm. The supernatant was discarded and the protoplasts resuspended in GUS extraction buffer (Jefferson *et al.*, 1987) for preparation of β-glucoronide extracts. The tubes were vortexed for 1 minute and then spun at 13000rpm for 2 minutes. The supernatant was collected and placed

in a fresh eppendorf tube. 20µl of the extract were used in the GUS assays. Fluorometric assays for GUS activity were performed with the substrate 4-methylumbelliferyl-D-glucuronide (Sigma™) using a Perkin-Elmer™ LS-35 fluorometer (Jefferson *et al.*, 1987). Protein concentration of tissue homogenates were determined by the Bio-Rad™ protein assay (Bradford, 1976).

Example 2. Transient expression of enhanced LuxR.

The transient expression experiments were carried out as described above. The same reporter plasmid was used but different effector constructs were produced. In order to address whether the presence of a strong activator such as VP16 would enhance transcriptional efficacy of the receptor, it was introduced in both the N- and C- terminal ends of *LuxR*. The VP16 was also fused to the nuclear localisation signal (NLS) from SV40. Both the NLS of SV40 and VP16 were obtained from the yeast two hybrid plasmid pPC86 as a fused fragment which meant that it could be fused as one on to *LuxR*. The N-terminal fusion was constructed by isolating and introducing into pBluescript a *ClaI/NotI* fragment of pPC86 containing the SV40 NLS and Gal4 activation domain. The resulting pBluescriptSV40V was digested with *Hind*III and filled in followed by *Bgl*II digestion. The fragment was introduced into *Sma*I/*Bam*HI pDH51luxR vector (*Bam*HI and *Bgl*II enzymes produce compatible ends) to produce the N-terminal fusion in *LuxR* and yield plasmid pDH51NVLuxR (Figure 10). The methionine start site is provided by the SV40 fragment.

The C-terminal end fusion was carried out by placing at the *Rca*I site in LuxR the blunted fragment of NLS/VP16. pDH51LuxR was digested with *Rca*I, blunt ended and ligated to the *ClaI/NotI* SV40-Gal4 blunt ended fragment. The fusion resulted in plasmid pDH51LuxRNV which contains four linking amino acids, C A K L, between the end of LuxR and the start of the nuclear locating sequence.

The plasmids were tested in tobacco mesophyll protoplasts as described in Example 1, where p221.9lux6 was introduced with or without either of the expression vectors containing the enhanced luxR. GUS assays were carried out as described above.

Example 3. Transient expression of LuxR, LasR and TraR in monocot protoplasts.

Expression plasmid construction.

The vector of choice for expression in monocot transient system was pFun which contains ubiquitin promoter region and the Nos terminator. In between the two there is multiple unique cloning sites. LuxR was transferred into pFun as a BamHI fragment. pLasR was transferred as a BamHI/KpnI fragment. TraR was isolated from *Agrobacterium tumefaciens* by PCR using oligonucleotides TraR1 5' AATTGGTACCCACCATGCAGCACTGGCTGGACAAGTTGACC 3'(Sequence ID. 11) and TraR2 5' AATTGGATCCCAGATCAGCTTTCTTCTGCTTGGCGAGG 3' (Sequence ID 12). The purified fragment was restriction enzyme digested with BamHI and introduced into pFun BamHI vector. On each case the expression vectors were named as follows: pFunLuxR (Figure 13), pFunLasR (Figure 14) and pFunTra (Figure 15).

Reporter plasmid construction

Reporter plasmid used with the expression vector encoding for LuxR is the same as that used in the transient experiments carried out in Tobacco (Examples 1 and 2). In the case of reporter vectors for both the expression of LasR and TraR the parental plasmid p221.9 was used. In both cases oligonucleotides encoding the LasBox and the two TraBoxes were synthetically made. The LasBox oligonucleotides are as follows LasBox1 5' TCGACACCTGCGAGTTCTCCGAGGTG 3' (Sequence ID 13) and LasBox2 is 5' TCGACACCTCGGAGAACTCGCAGG TG 3' (Sequence ID 14). The TraBoxes as described by Zhu and Winans, (1999) were used and the oligos are as follows, TraBox1 5 ' TCGACTACACGTCTAGACGTGTAGG 3' (sequence ID 15), TraBox2 5' TCGACCTACACGTCTAGACGTGTAG 3'(Sequence ID 16) (first pair), TraBox3 5' TCGACTACACGTCTAGACGTGTAAG 3' (Sequence ID 17) and trabox4 5' TCGACTTACACGTCTAGACGTGTAG 3' (second pair) (Sequence ID 18). Equimolar amounts of each pair oligo in the pair were mixed denatured and allow to cool down slowing to form double stranded DNA which had SalI cohesive ends. The double stranded DNA was then ligated into a SalI digested p221.9 vector. Recombinants were screened by colony hybridisation and

sequenced to ascertain number of elements incorporated into the vector. The vectors were named as follows p221.9Trabox1 (Figure 16), p2219Trabox2 (Figure 17) and p221.9Lasbox (Figure 11).

Example 4. Stable expression of LuxR in Tobacco plants

Binary vector construction

The reporter cassette was isolated from p221.9lux6 by digesting with *Eco*RI and *Hin*dIII to yield a 2.0kb fragment. The fragment was purified and introduced into pBin 19 *Eco*RI/*Hin*dIII vector to produce pBinrepAHL. The three different variants of the *LuxR* receptor, that is *LuxR* and the two enhanced versions were restriction enzyme digested with *Eco*RI (site flanking both sides of the effector cassette) and introduced into a dephosphorylated *Eco*RI pBin19repAHL vector to produce either pAHL1 (LuxR) (Figure 18), pAHL2 (SVLuxR) (Figure 19) or pAHL3 (LuxRSV) (Figure 20).

Plant transformation

The plant transformation construct pAHL1 (Figure 18), pAHL2 (Figure 19) and pAHL3 (Figure 20), containing *LuxR* or chimeric *LuxR* and a reporter gene cassette, were transformed into *Agrobacterium tumefaciens* LBA4404 using the freeze/thaw method described by Holsters *et al.* (1978). Tobacco (*Nicotiana tabacum* cv Samsun) transformants were produced by the leaf disc method (Horsch *et al.*, 1988). Shoots were regenerated on medium containing 100mg/l kanamycin. After rooting, plantlets were transferred to the glasshouse and grown under 16 h light/ 8 h dark conditions

PCR analysis

Analysis of transgenic tobacco plants by PCR was carried out using leaf sample extracted in 300µl of extraction buffer. The DNA was precipitated with isopropanol at 4°C for 10 minutes and then centrifuged. The pellet was dried and resuspended in 100µl of TE (10mM Tris HCl pH 8.0, 1mM EDTA). 2.5 µl were placed in a 500 µl eppendorf tube and a master mix containing buffer, dNTPs and oligonucleotides was

added. The *Taq* polymerase (Gibco-BRL™) was added after samples were denatured for 3 minutes.

Chemical treatments

OHHL was dissolved in methanol (Sigma) and the stock maintained at -20°C. The compound was diluted in growth media used to germinate seedlings. Uninduced seedlings were treated with equivalent amount of methanol. Seeds were germinated in MS media supplemented with 0.8% (w/v) agar. Seedlings were collected 2 days post-germination (two cotyledon stage).

GUS Assay

In seedling induction experiments 10 two day old seedlings were collected and flash frozen in liquid nitrogen. The seedlings were homogenised in 300 µl of GUS extraction buffer and centrifuged for 5 minutes at 13000rpm. The supernatant was used for both GUS and Bio-Rad™ protein assays.

Example 5 Stable constitutive expression of *yenI* gene in tobacco plants

Binary vector construction.

pBDHELI (Figure 21) was constructed by fusing the alfalfa mosaic virus (AMV) translation enhancer sequence from pBi526 (Datla et al., Plant Science 94, 139-140 (1993)) to the *yenI* coding sequence from *Yersinia enterocolitica*. AMV-*LuxI* gene fusion was directional cloned into pDH51 (Pietrzak *et al.*,1986) vector to produce pDHELI. A 1.8kb fragment of pDHELI was cloned into pBin19 (Bevan, 1994) to give pBDHELI (Figure 21). The plasmid was introduced into *Agrobacterium* as described above. A tobacco plant population of was produce and screened as detailed above. A high expressor of *yenI* gene was selected by their ability to synthesised OHHL. The assay consists of placing a leaf of the transgenic plant on an agar plate overnight. The leaf was then removed and the *cviI* mutant of *Chromobacterium violaceum* spread over the plate. Violacein (a purple pigment) production by the bacterium can be seen when OHHL had diffused out of the leaf and into the agar (Figure 24).

Example 6. LuxR is activated by compounds produced by *yenI* plants.

The root of a transgenic tobacco plant containing the *yenI* expression cassette, that was grown in tissue culture under aseptic conditions, was placed on an LB agar plate and overlain with top agar containing *E. coli* with the plasmid pSB401 (Figure 22). This strain expresses the *lux* operon (i.e. *luxR*, *lux* ABCDE) and will bioluminesce in response to OHHL and HHL. Figure 24 shows that acyl-homoserine lactones produced by *yenI* introduction into tobacco plants are capable of activating *LuxR* harboured in *E .coli* and activate reporter gene expression. These data suggest that introduction of *LuxR* expression and reporter cassettes into plants harbouring the *yenI* gene will result in activation of reporter gene expression.

Example 7. Cross of *LuxR* tobacco high expressor plant with *yenI* tobacco plant.

Plants are produced by crossing the *yenI* expressing plant with the AHL switch plants. The seed of the progeny was collected and assayed for GUS activity as described above. GUS activity was assayed for in all tissues and different ages as the expectation was that GUS protein would be present in all tissues expressing the *yenI* gene. *yenI* is under control of the 35S CaMV promoter as is the *LuxR* protein in the AHL switch. Initially, progeny seedlings were grown in 1/2MS and were collected 2 days post-germination or when cotyledons were fully extended.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the invention.

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CLAIMS

1. A method of initiating transcription of a target gene in a eukaryotic cell comprising:

(a) providing a eukaryotic cell which is capable of producing a response protein;

(b) inserting into the genome of said cell a polynucleotide defining an inducible promoter sequence operably linked to and capable when induced of initiating transcription of said target gene; and

(c) applying to said cell a chemical inducer capable of binding to said response protein whereby said chemical inducer binds to said response protein to form an inducing complex which binds to and induces said inducible promoter thereby initiating transcription of said target gene.

2. A method according to claim 1 wherein a polynucleotide is inserted into the genome of said eukaryotic cell which polynucleotide provides for the production of the said response protein.

3. A method according to claim 1 or 2 wherein the inducible promoter comprises the nucleotide sequence depicted as SEQ ID No. 2 and the response protein comprises the amino acid sequence depicted in SEQ ID No. 16.

4. A method according to claim 3 wherein the said inducible promoter comprises a polynucleotide which is the complement of one which binds to SEQ ID No. 2 at a temperature of between 60°C and 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS wherein the said polynucleotide is still capable of acting as an inducible promoter upon binding with the said inducing complex.

5. A method according to claim 3 or 4 wherein the polynucleotide encoding the said response protein comprises the sequence depicted in SEQ ID No. 5.

6. A method according to claim 5 wherein the polynucleotide encoding the said response protein comprises the complement of one which binds to SEQ ID No. 5 at a temperature of between 60°C and 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS wherein the said polynucleotide still encodes a protein which is capable of forming an inducing complex with the said chemical inducer.

7. A method according to any one of claims 2 to 6 wherein the polynucleotide encoding the said inducible promoter contains the region depicted as SEQ ID No. 21.

8. A method according to any one of claims 1 to 7 wherein the said eukaryotic cell is a plant cell.

9. A method according to claim 8 wherein the plant cell is from a plant selected from the group consisting of: melons, mangoes, soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, peaches, grape, strawberries, carrot, lettuce, cabbage, onion, citrus or nut plants.

10. A method according to any one of claims 1 to 9 wherein the said chemical inducer is N-(3-oxo)hexanoyl-L-homoserine lactone or a functional equivalent thereof.

11. A method according to any one of claims 8 to 10 wherein the said chemical inducer is applied to the plant cell as part of an agriculturally acceptable formulation.

12. A method according to any one of claims 2 to 11 wherein the polynucleotide encoding the said response protein is bounded by a promoter and a terminator sequence.

13. A method according to claim 12 wherein the promoter is inducible.

14. A method according to claim 13 wherein the promoter is selected from the group consisting of: Alc A/ R switch system, the GST switch system and the ecdysone switch.

15. A method according to claim 12 wherein the promoter is constitutive or developmentally regulated.

16. A method according to claim 15 wherein the promoter is selected from the group consisting of: Cauliflower mosaic virus 35S/19S, maize Ubiquitin and *Arabidopsis* Ubiquitin 3.

17. A method according to any one of claims 1 to 16 wherein the said target gene encodes β-glucuronidase; *Bacillus thuringenesis* toxin; barnase or barstar.

18. A method of providing plants containing an inducible target gene comprising:

(a) inserting into a plant cell which cell provides for production of a response protein a polynucleotide defining an inducible promoter sequence operably liked to said target gene; and

(b) regenerating morphologically normal fertile plants thereof; and

(c) applying to the population of regenerants a chemical inducer or a functional variant thereof capable of binding to said response protein, whereby said chemical inducer binds to said response protein to form an inducing complex which binds to and induces said inducible promoter thereby initiating transcription of said target gene; and

(d) selecting those plants which are expressing the said target gene.

19. A method according to claim 18 wherein the said inducible promoter sequence comprises the nucleotide sequence depicted as SEQ ID No. 2 or a functional variant thereof and the response protein comprises the amino acid sequence depicted as SEQ ID No 16 or a functional variant thereof and the said chemical inducer is N-(3-oxo)hexanoyl-L-homoserine lactone or a functional equivalent thereof.

20. A method according to claim 18 wherein the said inducible promoter sequence contains the nucleotide sequence depicted as SEQ ID No. 10 or a functional variant thereof and the response protein comprises the amino acid sequence depicted as SEQ ID No 17 or a functional variant thereof and the said chemical inducer is N-(3-oxo)dodecanoyl-L-homoserine lactone or a functional equivalent thereof.

21. A method according to claim 18 wherein the said inducible promoter sequence contains the nucleotide sequence depicted as SEQ ID No. 12 or a functional variant thereof and the response protein comprises the amino acid sequence depicted as SEQ ID No 18 or a functional variant thereof and the said chemical inducer is N-(3-oxo)octanoyl-L-homoserine lactone or a functional equivalent thereof.

22. Plants produced according to a method of any one of claims 18 to 21.

23. Plants according to claim 22 which plants are selected from the group consisting of: melons, mangoes, soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, peaches, grape, strawberries, carrot, lettuce, cabbage, onion, citrus or nut plants.

24. A DNA construct which comprises a first polynucleotide region which comprises an inducible promoter sequence operably liked to and capable when

induced of initiating transcription of a target gene and a second polynucleotide region which provides for the production of a response protein wherein upon contact of the said response protein with a chemical inducer the said response protein binds the chemical inducer to produce an inducer complex which binds the said inducible promoter sequence thereby initiating transcription of the said target gene.

25. A DNA construct according to claim 24 wherein the said first polynucleotide comprises the nucleotide sequence depicted as SEQ ID No. 2 or a functional variant thereof and the second polynucleotide region comprises a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 16 or a functional variant thereof.

26. A DNA construct according to claim 24 wherein the said first polynucleotide comprises the nucleotide sequence depicted as SEQ ID No. 10 or a functional variant thereof and the second polynucleotide region comprises a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 17 or a functional variant thereof.

27. A DNA construct according to claim 24 wherein the said first polynucleotide comprises the nucleotide sequence depicted as SEQ ID No. 12 or a functional variant thereof and the second polynucleotide region comprises a polynucleotide which encodes the amino acid sequence depicted as SEQ ID No. 18 or a functional variant thereof.

28. A DNA construct according to any one of claims 24 to 27 wherein the said second polynucleotide region comprises a promoter operably linked to the polynucleotide encoding the said response protein.

29. A DNA construct according to claim 28 wherein the said promoter is inducible.

30. A DNA construct according to claim 28 wherein the said promoter is constitutive or developmentally controlled.

31. Use of a DNA construct according to any one of claims 24 to 30 in the production of a plant containing a target gene of which the expression may be controlled by the application of a chemical inducer to the said plant.

32. A method of screening compounds in a bioassay comprising applying an amount of a chemical to a plant which plant contains a polynucleotide comprising an inducible promoter operably linked to a region encoding a reporter protein and which plant is also capable of producing a response protein and testing the said plant for the production of the said reporter protein.

33. A method of selectively controlling pests in a field which field comprises crop plants and pests wherein the plants are those obtained according to claim 18 and the said target gene encodes a target protein which is capable of controlling the said pests said method comprising applying to the plants an amount of a chemical inducer which is sufficient to bind to the said response protein to produce the said inducing complex which is capable of initiating transcription of the target gene which provides for the production of the target protein in an amount which is sufficient to control the said pests.

34. A method of providing a plant which contains a target gene which is inducibly controlled comprising:

(a) inserting into a first plant cell a polynucleotide comprising a first inducible promoter operably linked to a target gene and regenerating a first morphologically normal fertile plant therefrom;

(b) inserting into a second plant cell a polynucleotide comprising a promoter operably linked to a region encoding a response protein which is capable of binding to a chemical inducer to produce an inducing complex which is then capable of binding the said inducible promoter to allow for the

initiation of transcription of the said target gene and regenerating a second morphologically normal fertile plant therefrom;

(c) cross pollinating said first plant with the said second plant or said second plant with said first plant and harvesting the seed therefrom; and

(d) growing said seed and applying to the resultant plants an amount of said chemical inducer which provides an inducing complex capable of binding the said inducible promoter to allow for the initiation of transcription of the said target gene.

FIGURE 1

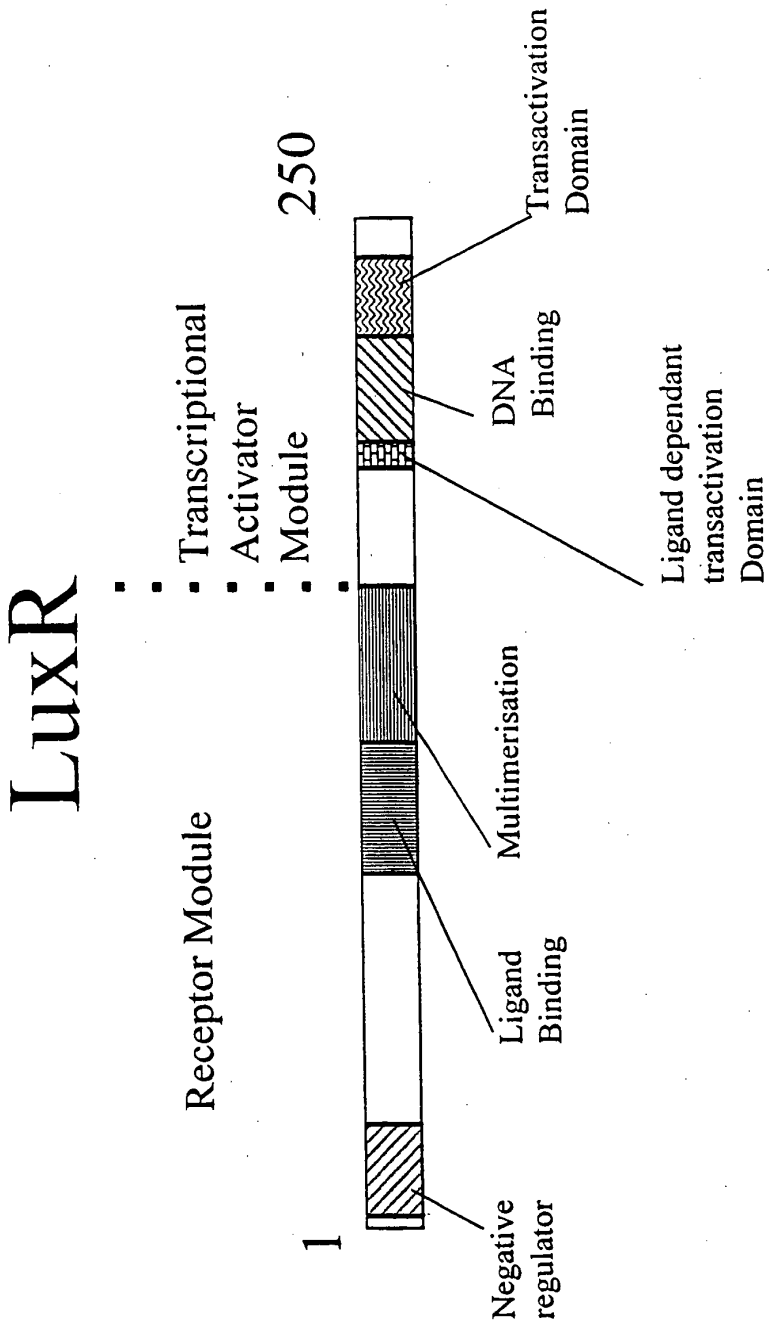


FIGURE 2

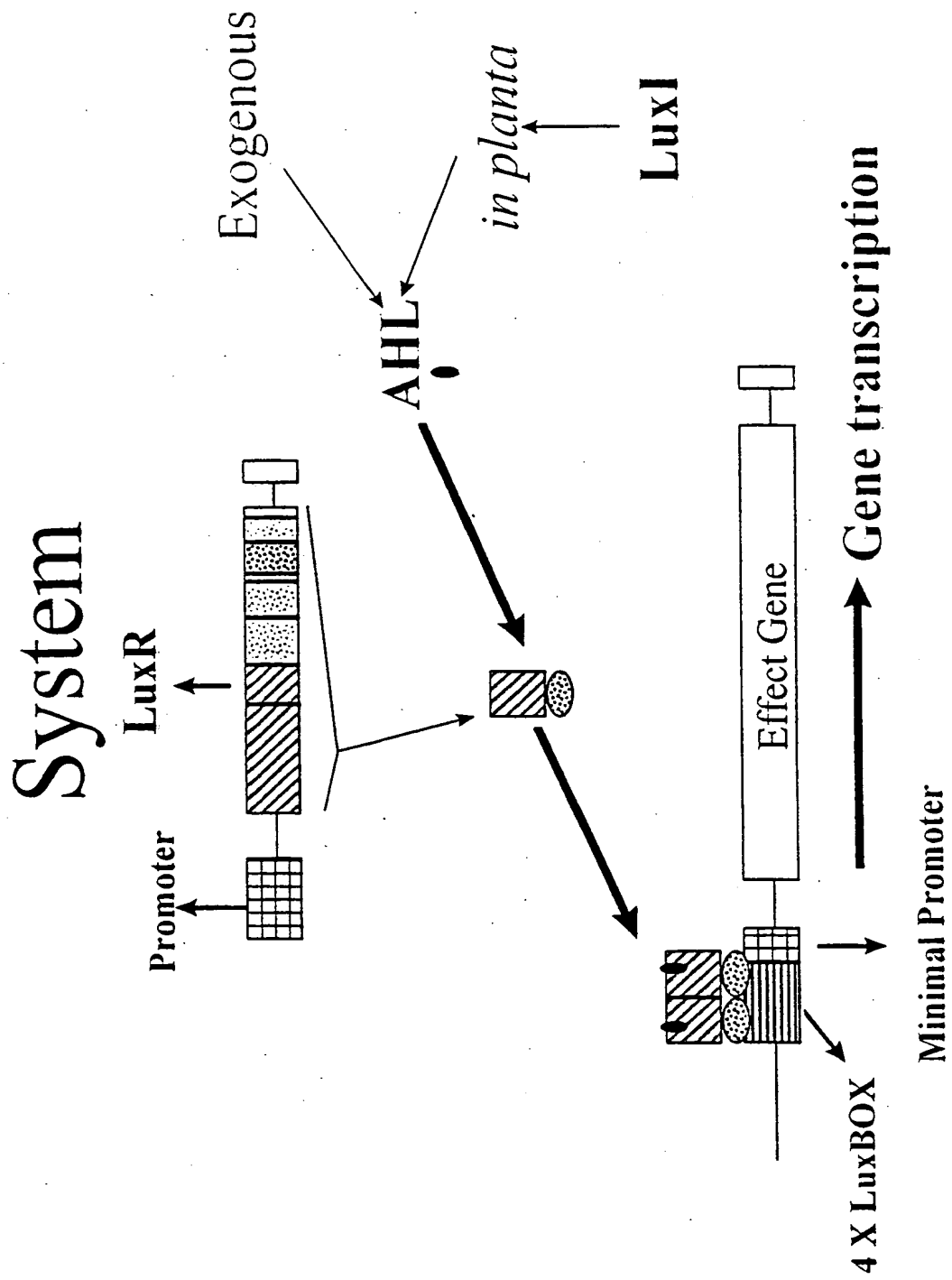

System
LuxR
Promoter
Exogenous
AHL
in planta
LuxI
Effect Gene
Gene transcription
Minimal Promoter
4 X LuxBOX

FIGURE 3

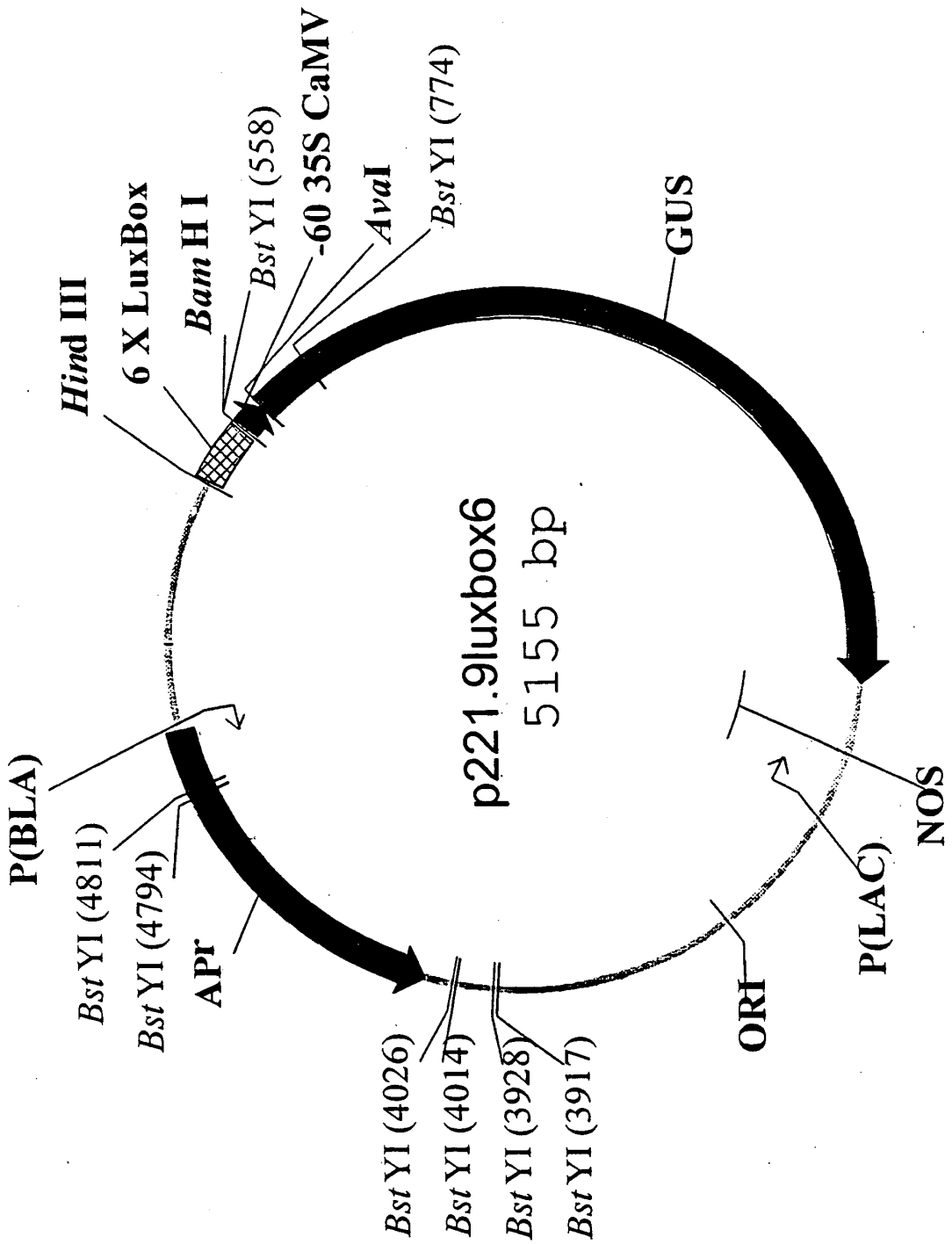


FIGURE 4

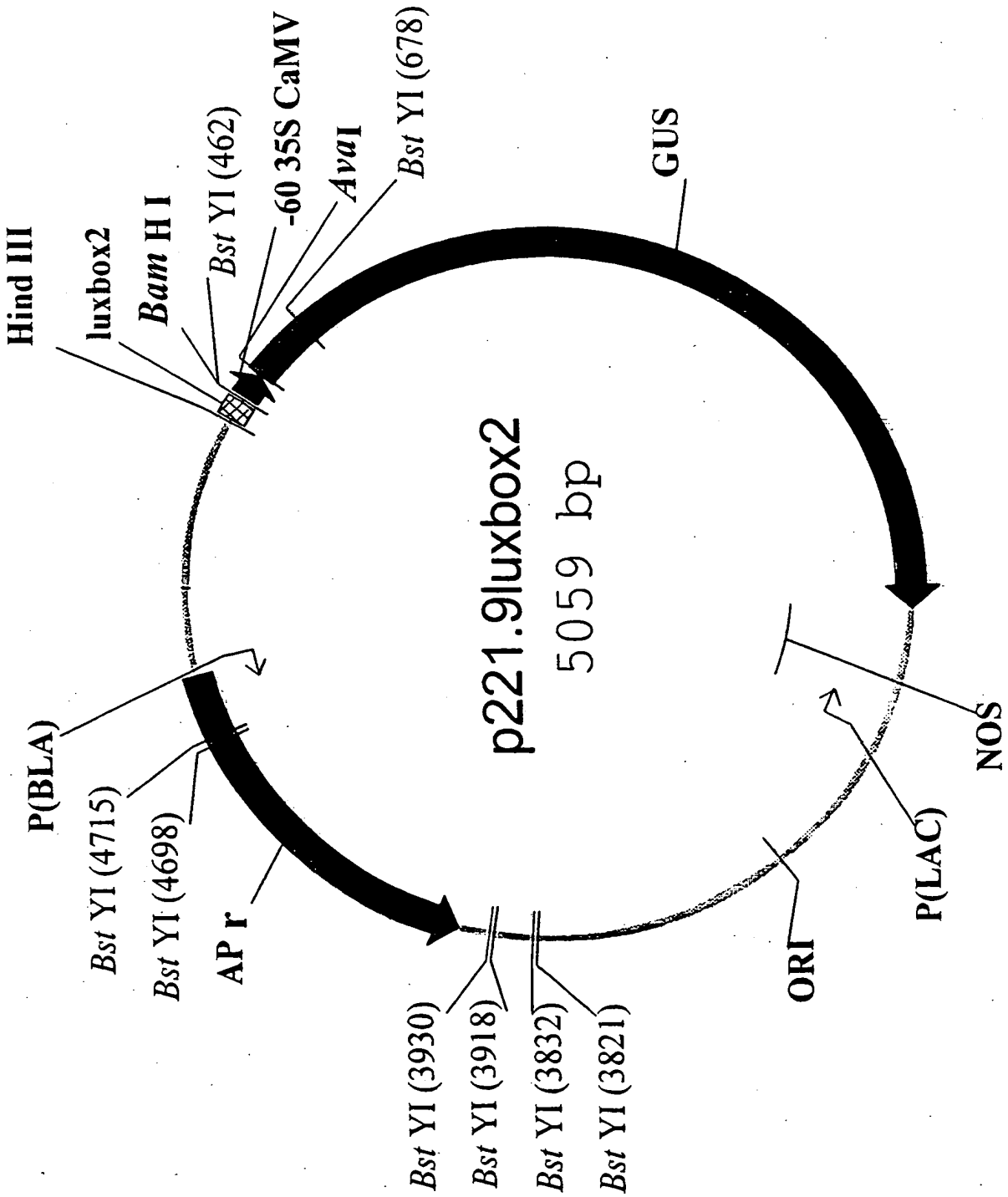
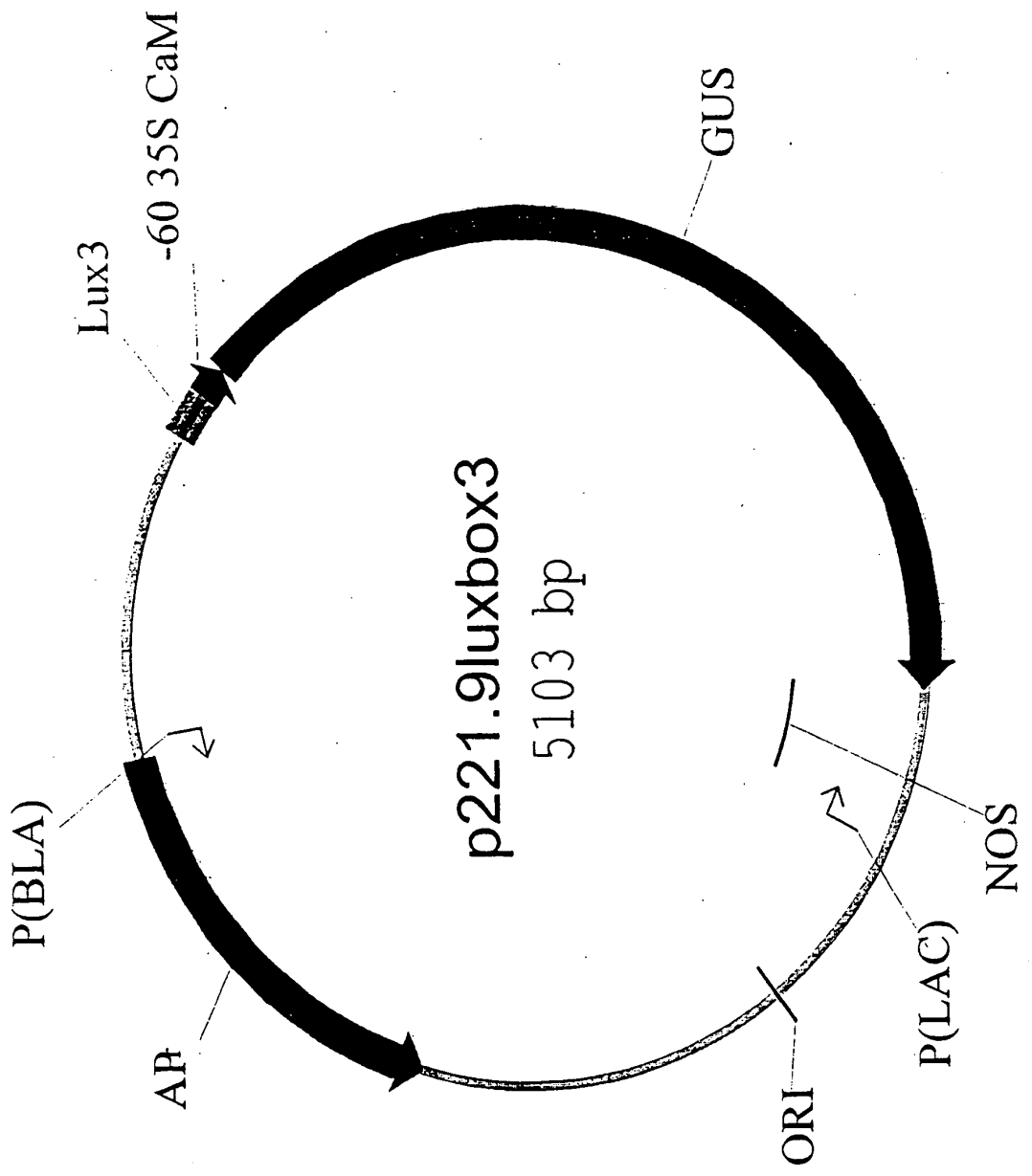


FIGURE 5



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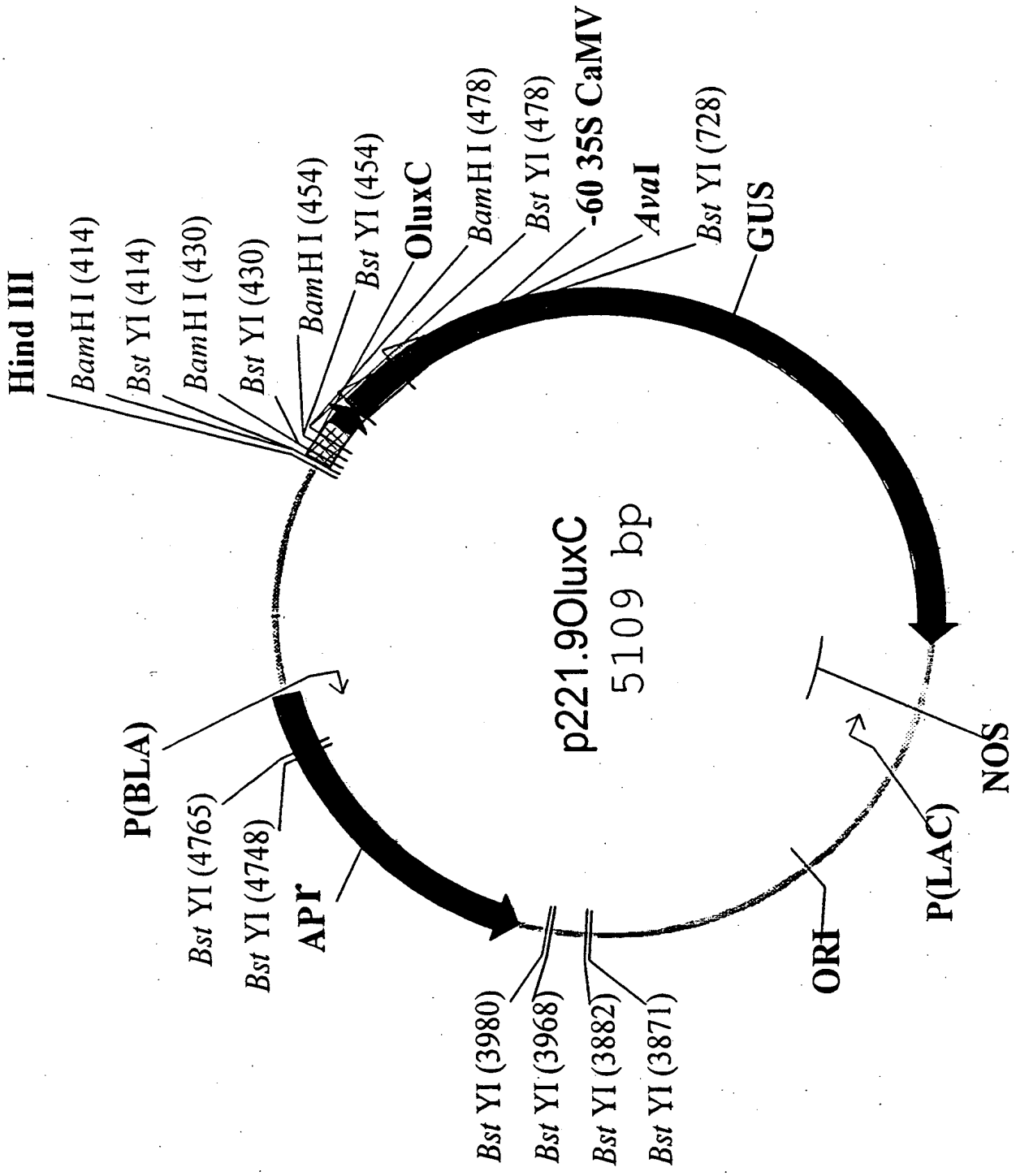


FIGURE 7

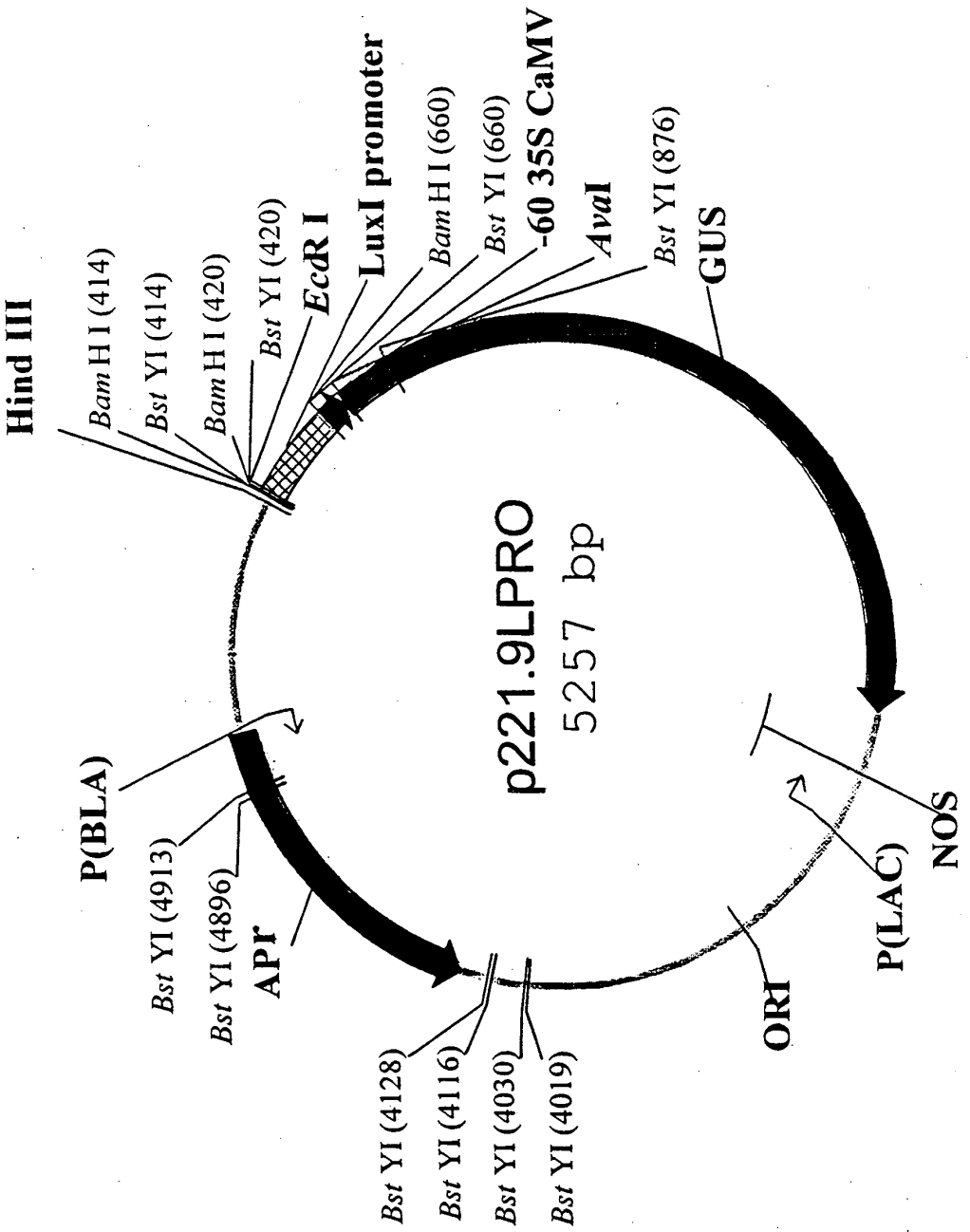
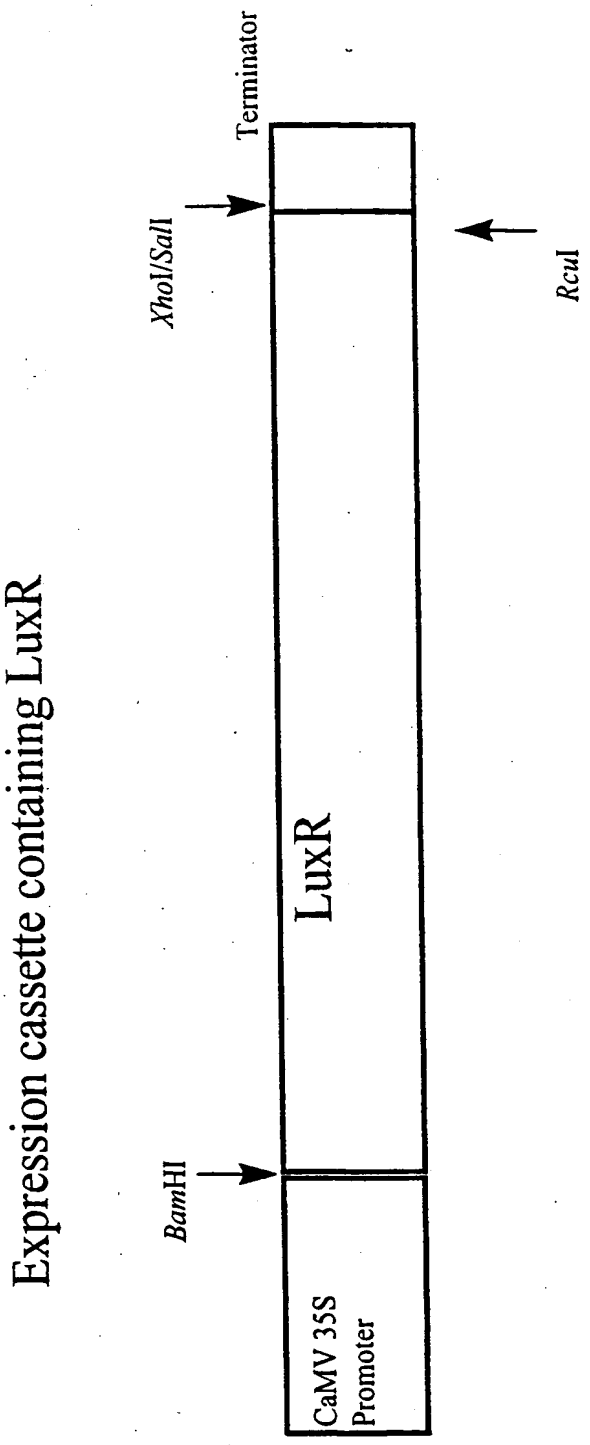


FIGURE 8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XhoI/SalI
RcuI
LuxR
BamHI
CaMV 35S
Promoter

FIGURE 9

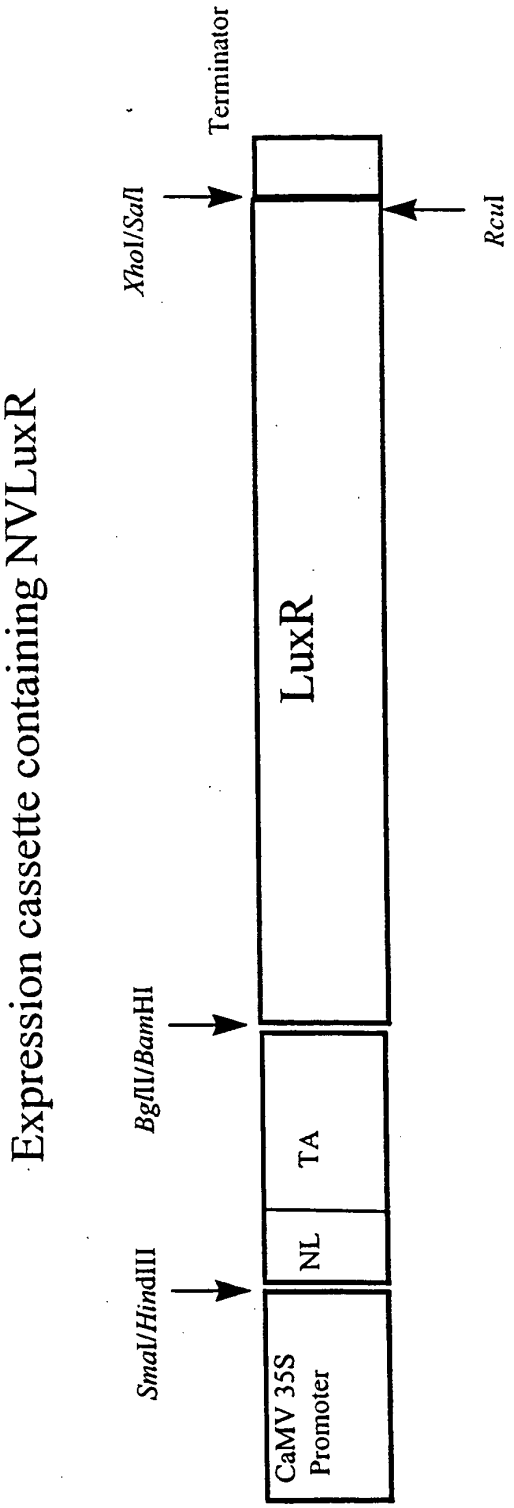
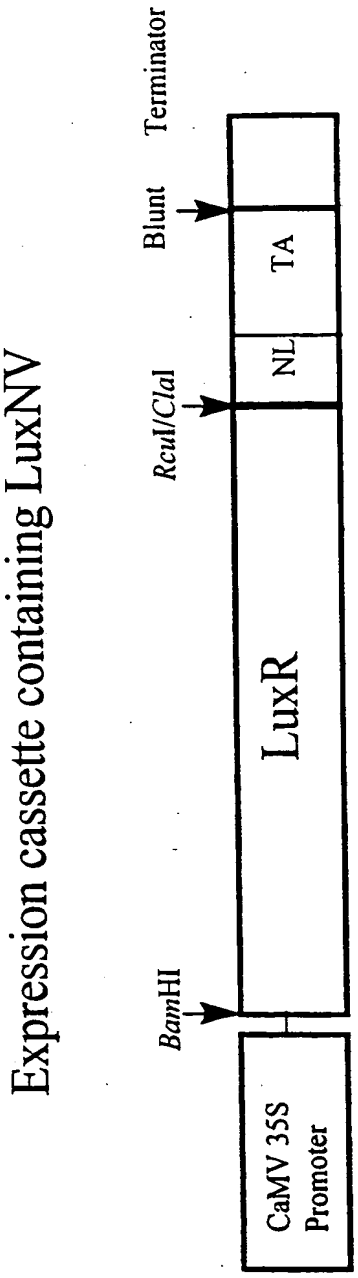


FIGURE 10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Blunt
TA
NL
RcuI/ClaI
LuxR
BamHI
CaMV 35S
Promoter

FIGURE 11

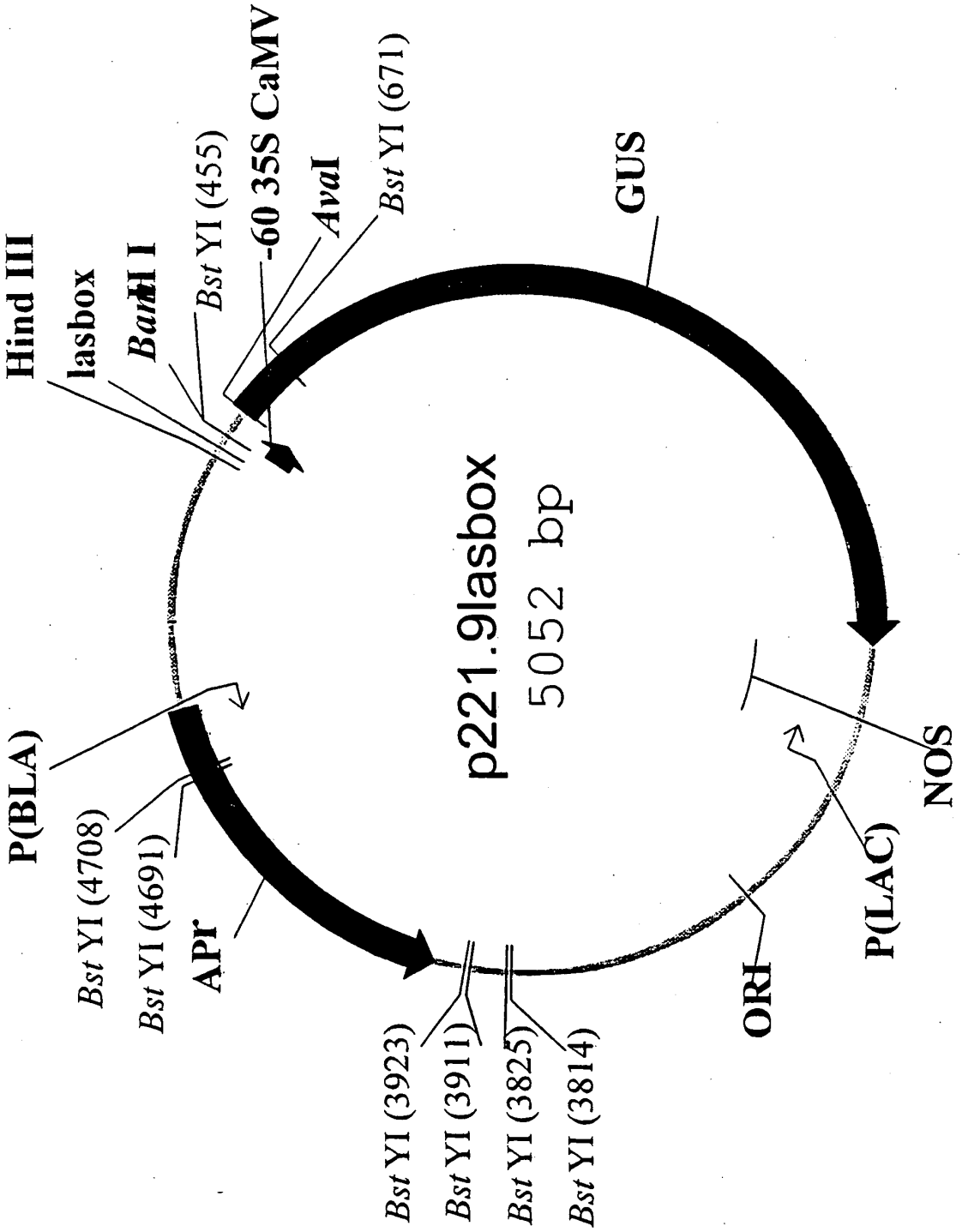

FIGURE 12

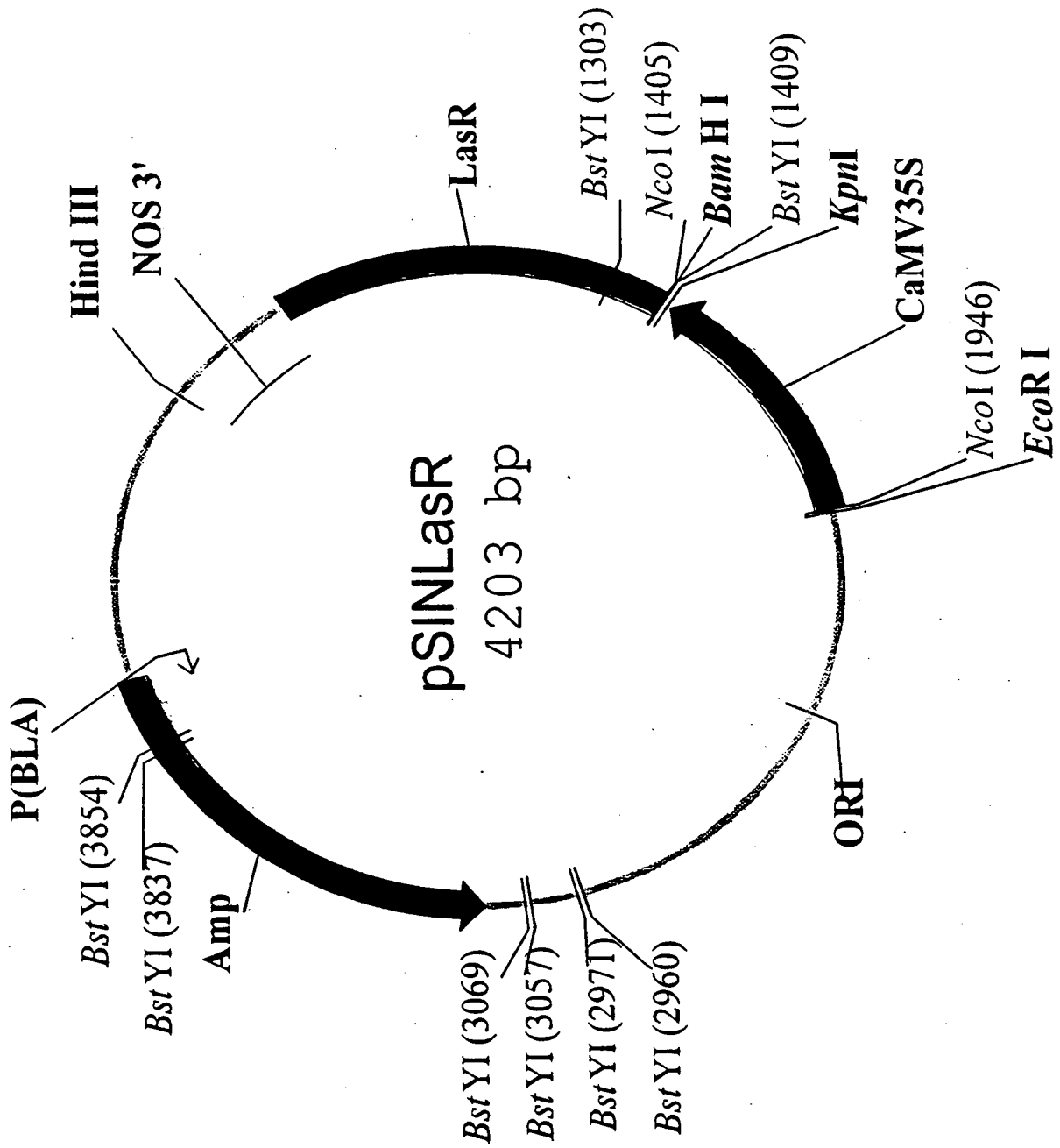

FIGURE 13

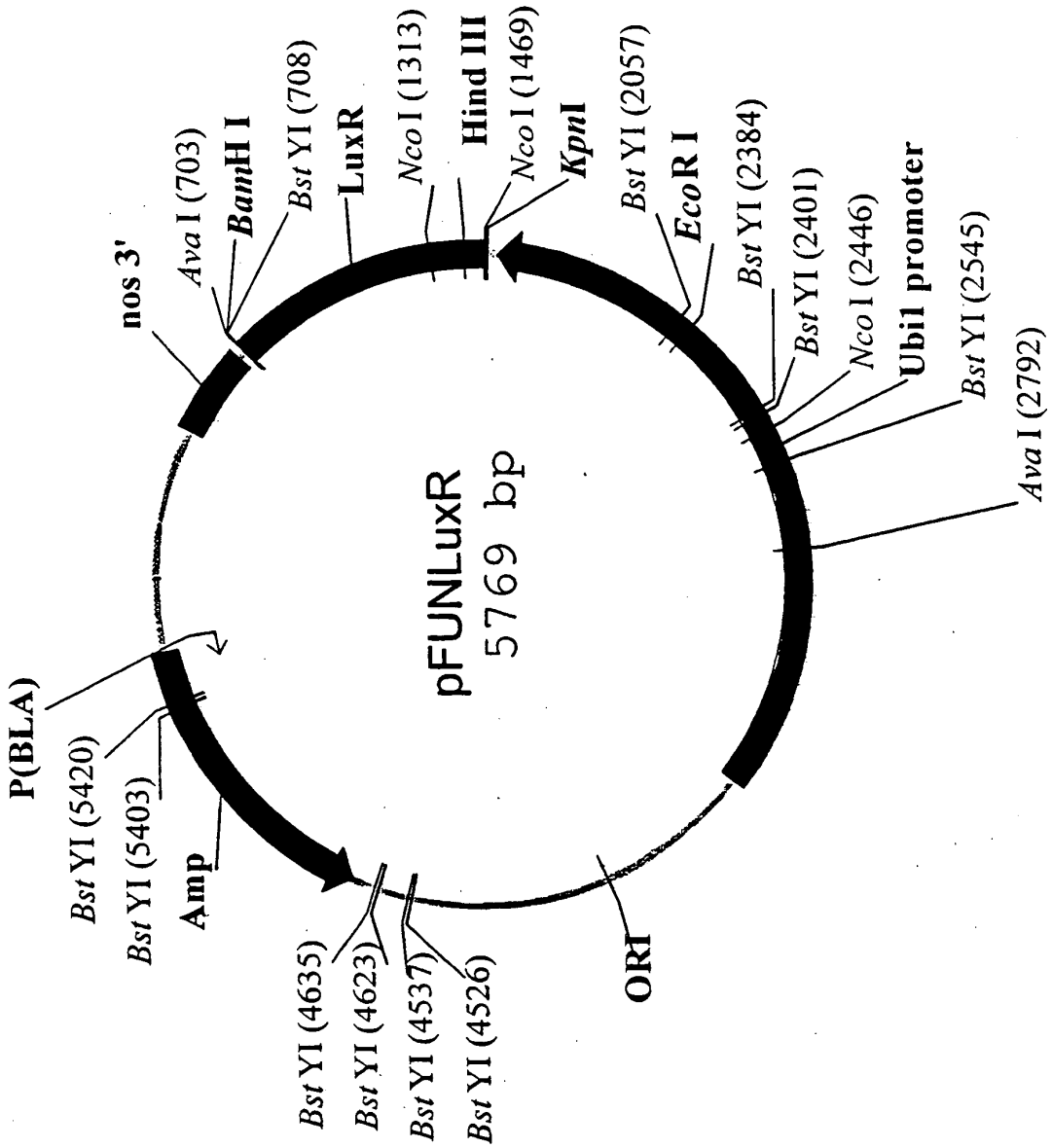

FIGURE 14

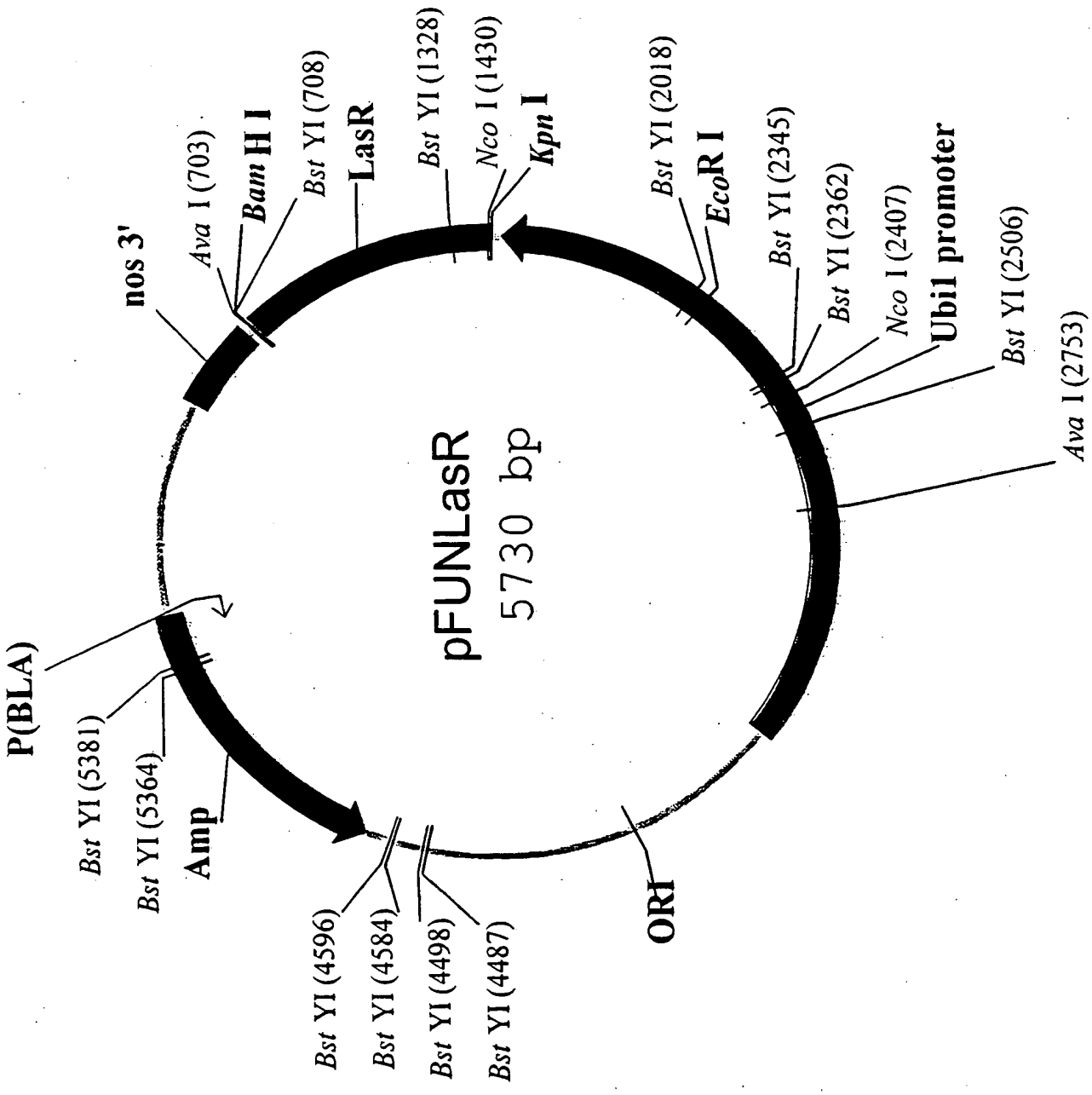

FIGURE 15

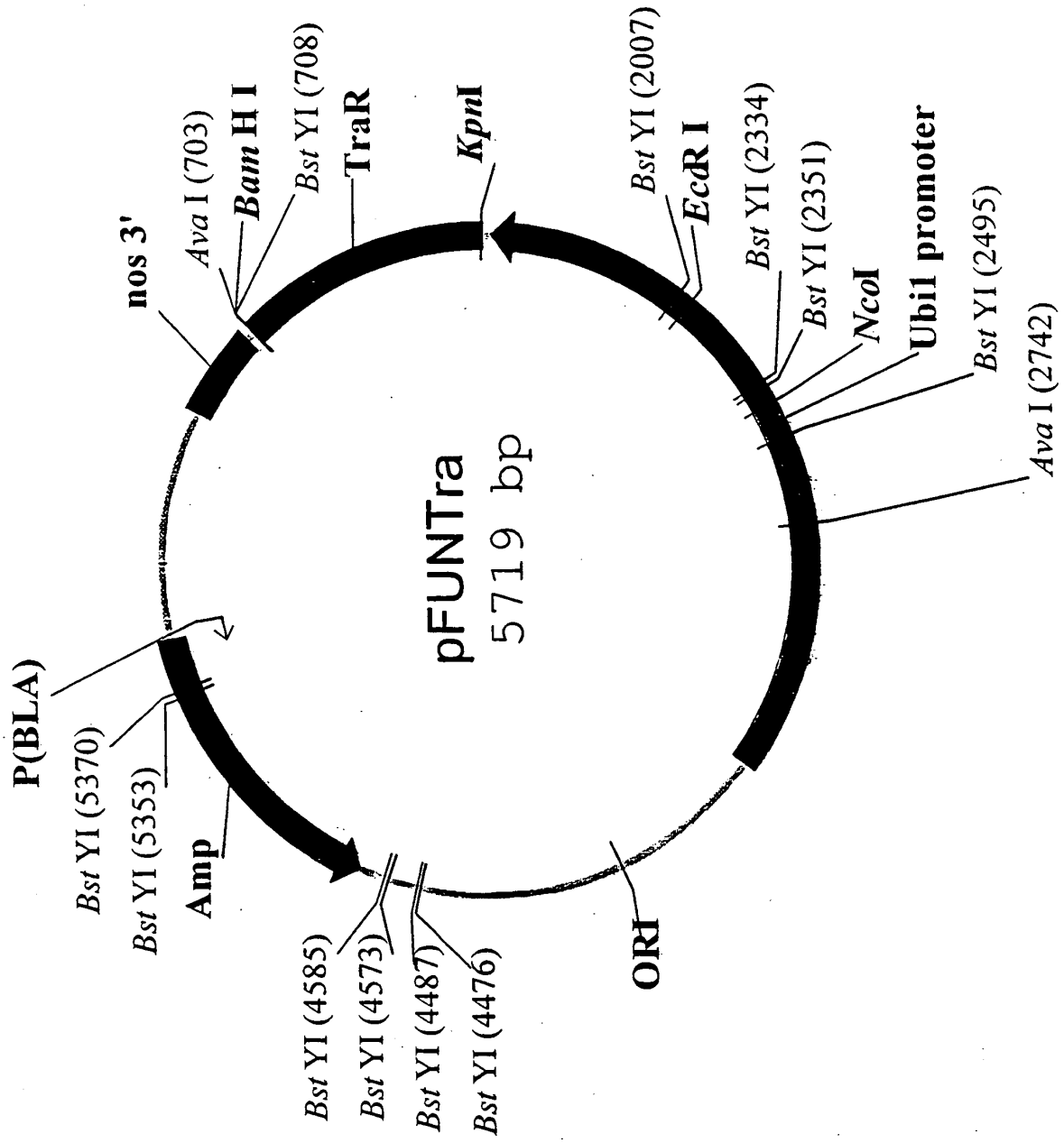

FIGURE 16

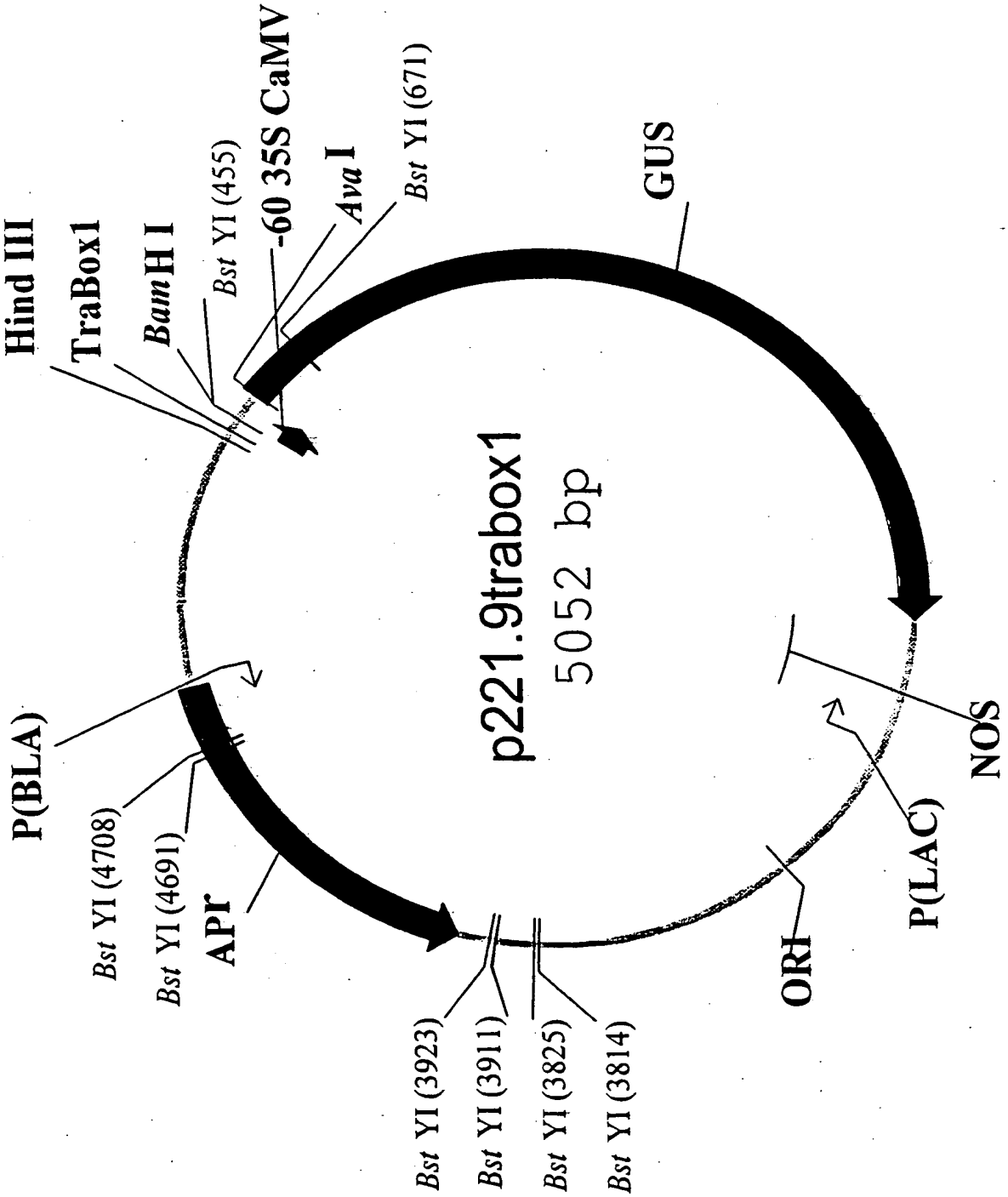

FIGURE 17

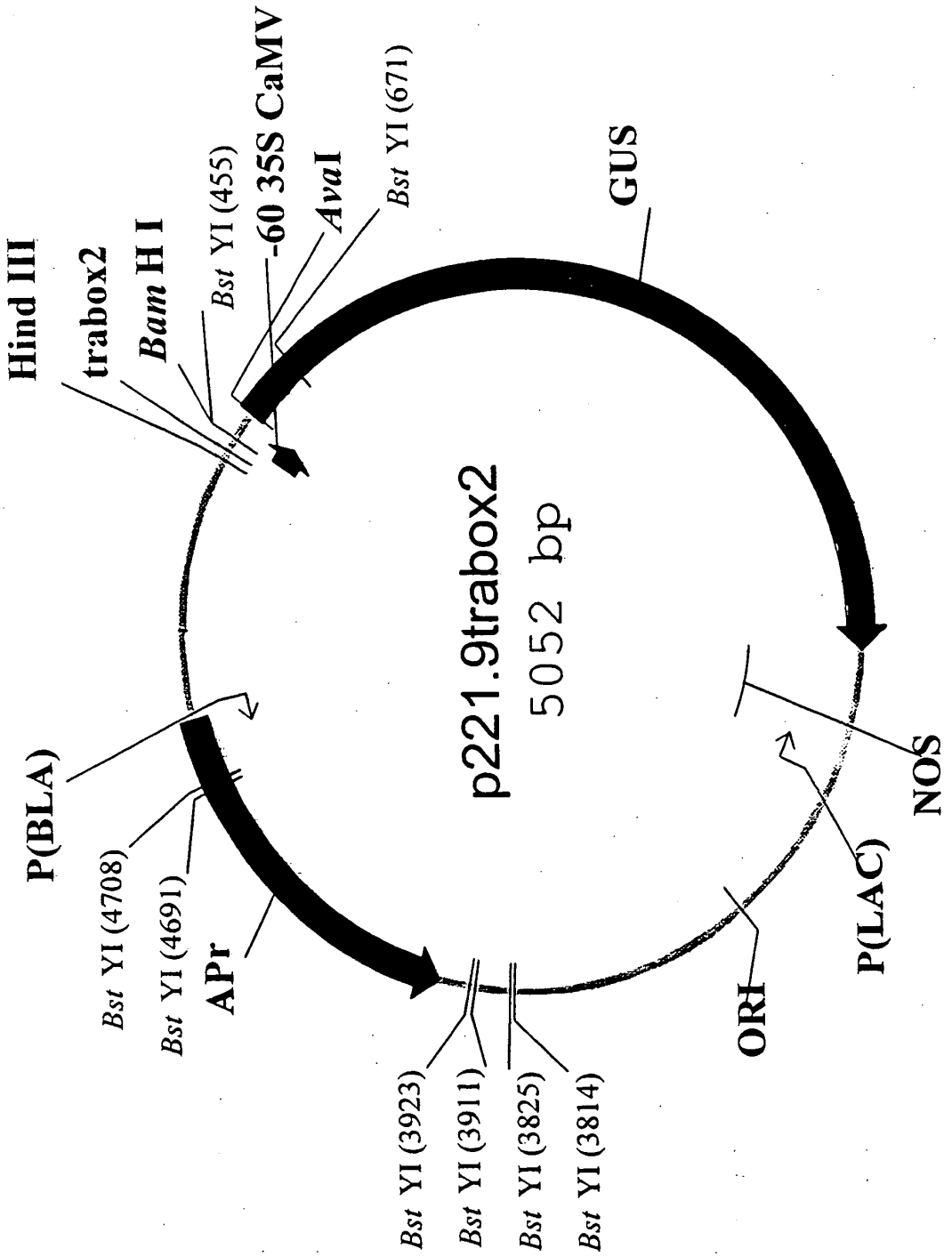

FIGURE 18

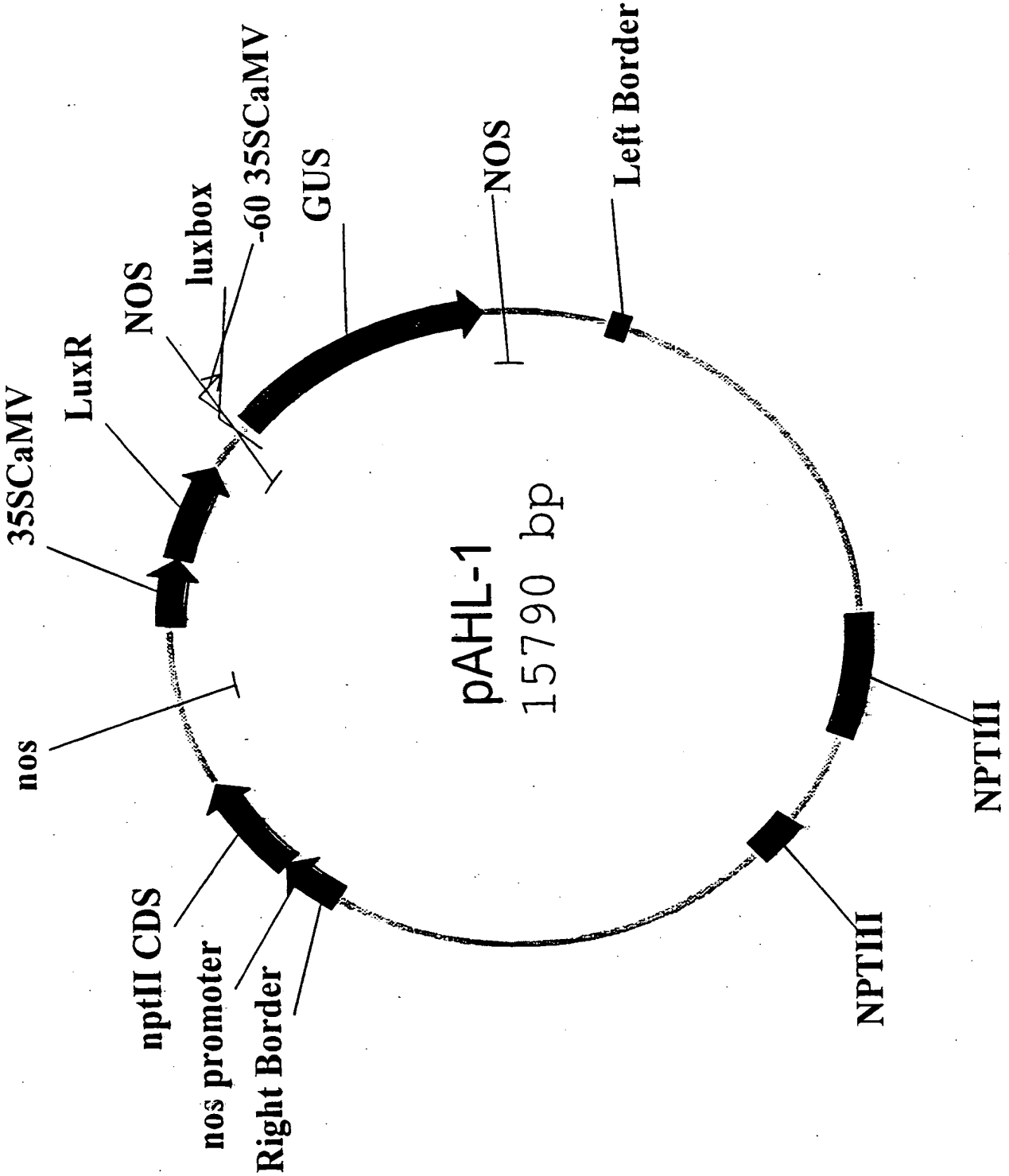

FIGURE 19

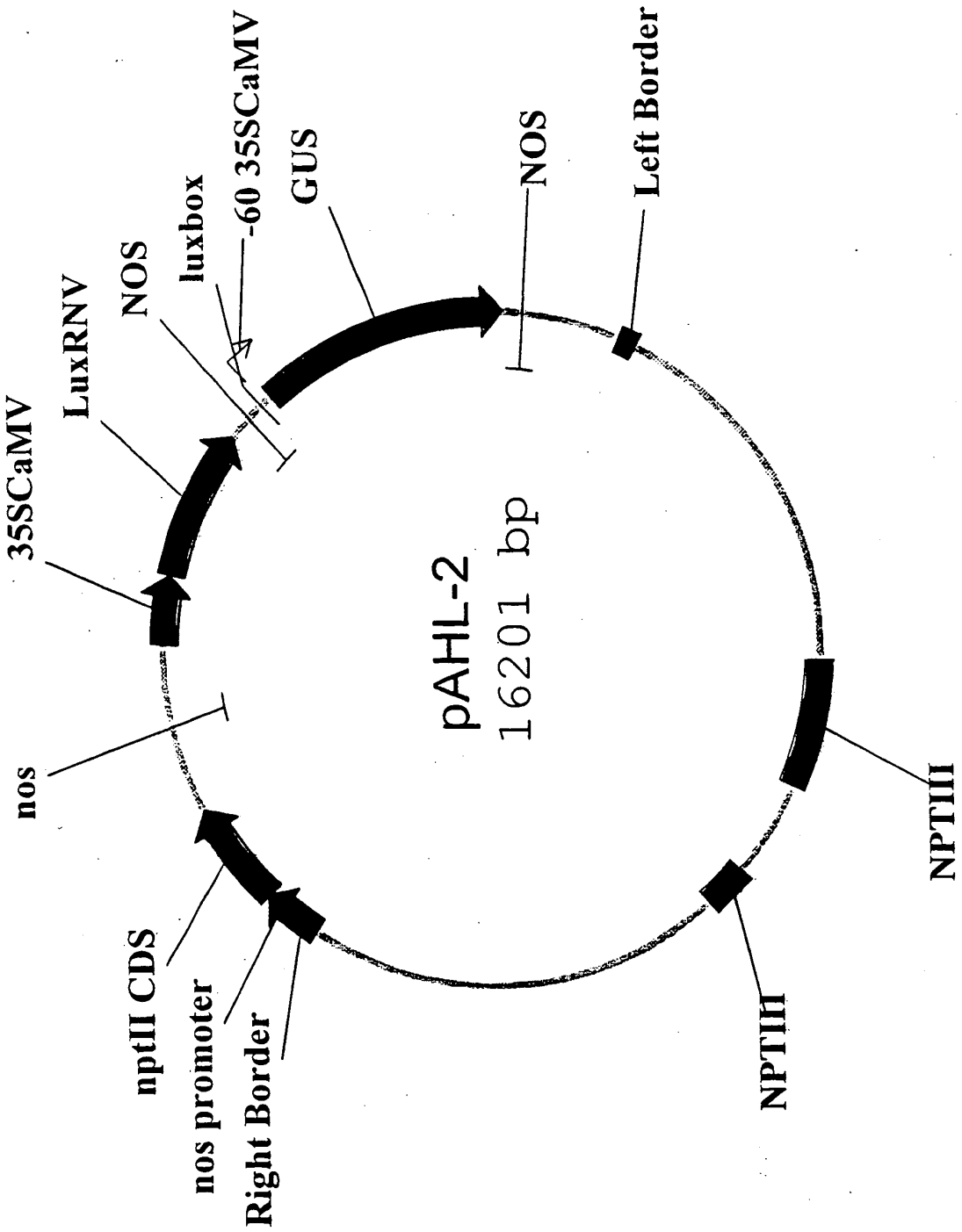

FIGURE 20

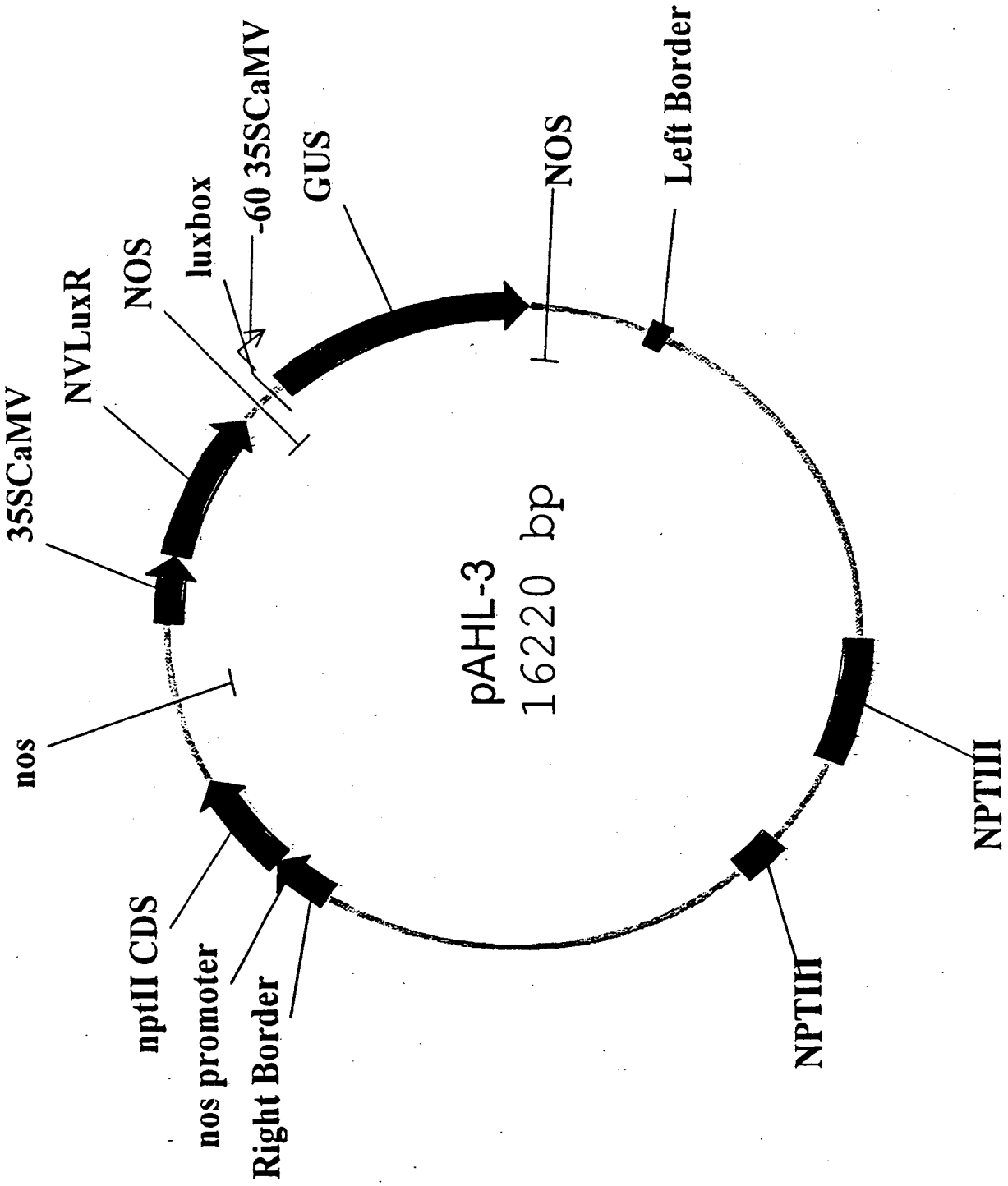

FIGURE 21

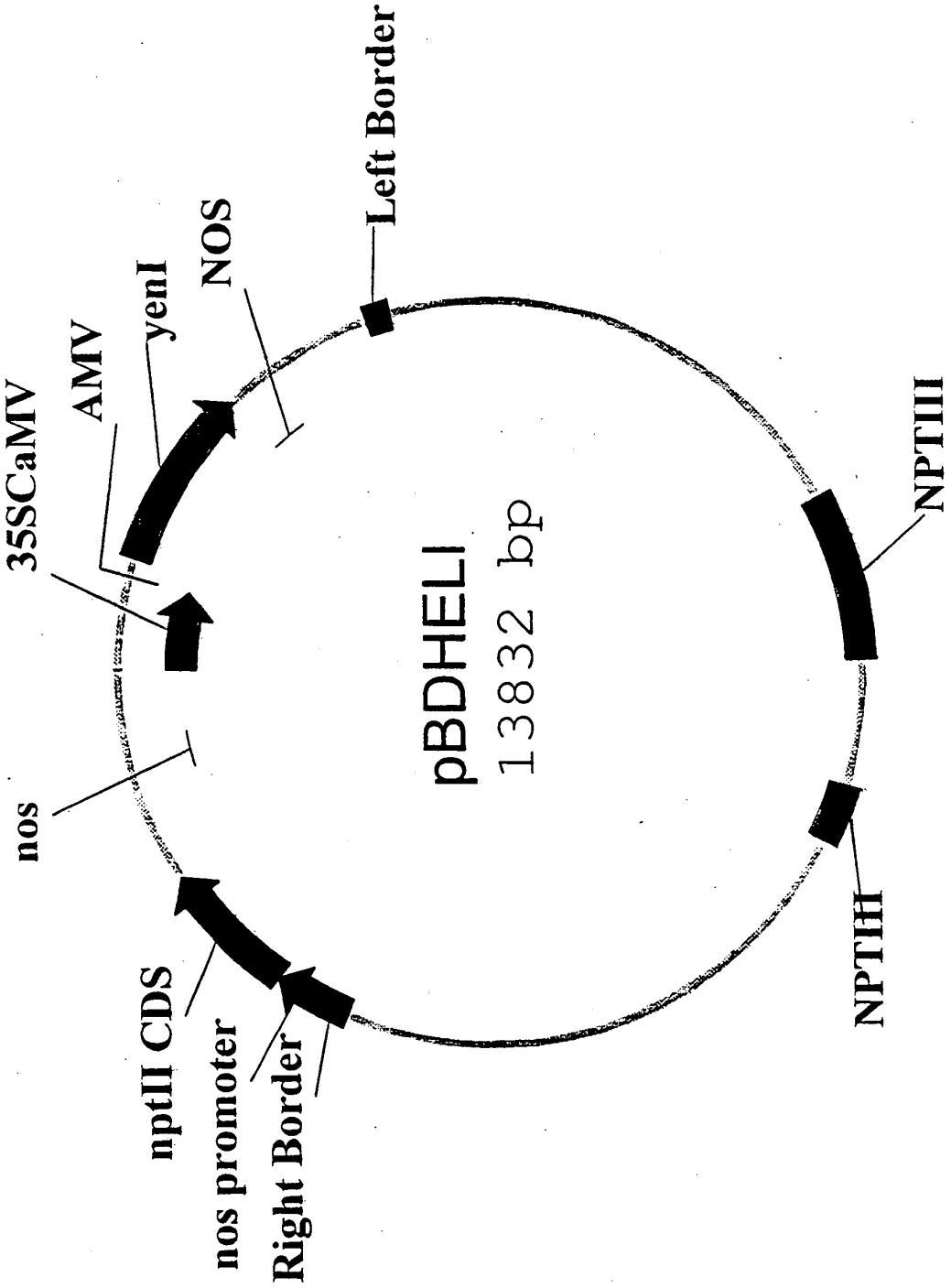

FIGURE 22

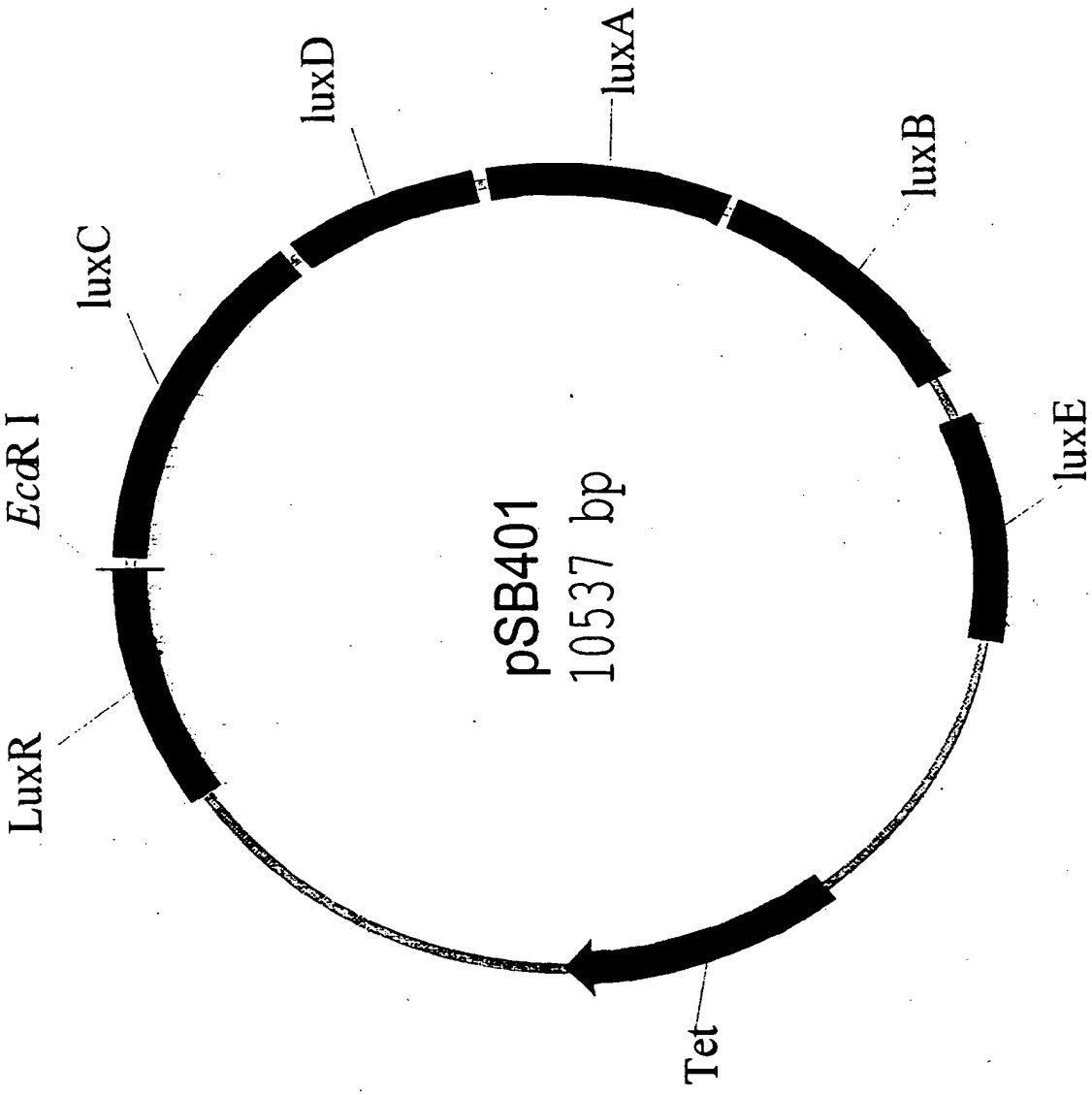

FIGURE 23

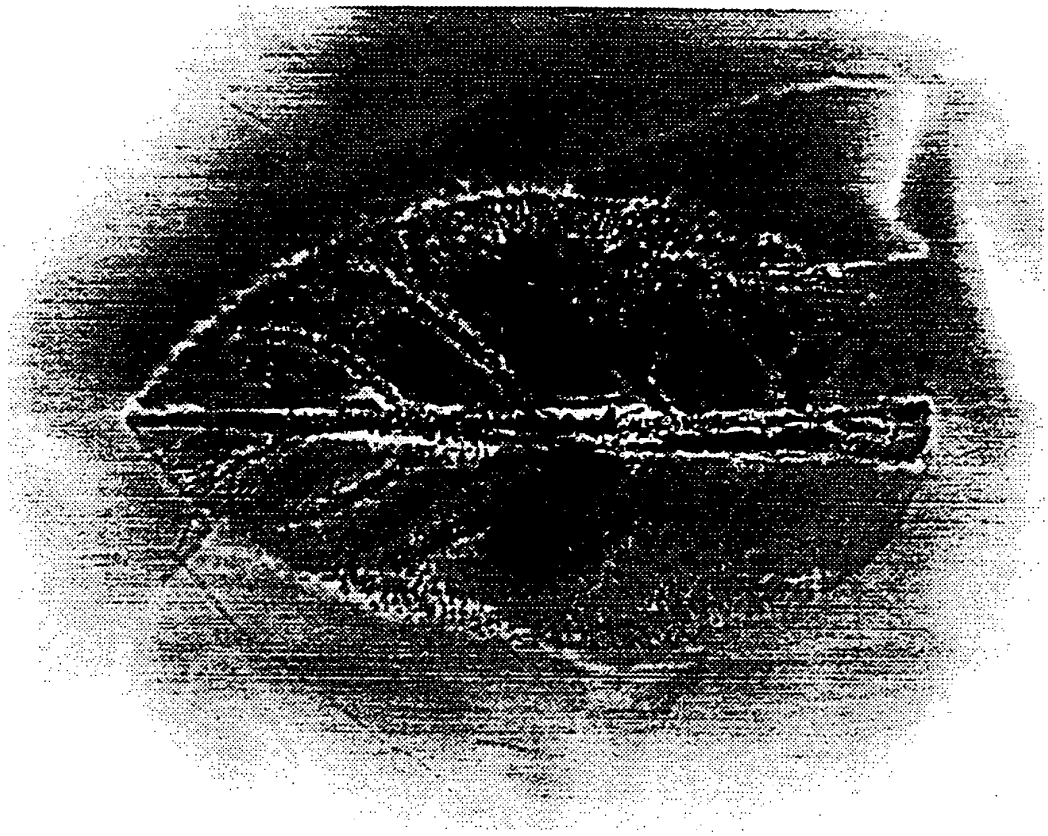

FIGURE 24

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65 70 75

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130 135 140

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145 150 155

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        35                  40                  45

Tyr Pro His Ser Met Val Lys Ser Asp Ile Ser Ile Leu Asp Asn Tyr
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Pro Lys Lys Trp Arg Gln Tyr Tyr Asp Asp Ala Asn Leu Ile Lys Tyr
65                  70                  75                  80

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                85                  90                  95

Asn Ile Phe Glu Asn Asn Ala Val Asn Lys Lys Ser Pro Asn Val Ile
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His Thr Ala Asn Asn Gly Phe Gly Met Leu Ser Phe Ala His Ser Glu
    130                 135                 140

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145                 150                 155                 160

Pro Leu Ile Val Pro Ser Leu Val Asp Asn Tyr Arg Lys Ile Asn Ile
                165                 170                 175

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Leu Gly Cys Ser Lys Arg Thr Val Thr Phe His Leu Thr Asn Ala Gln
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Thr Asn Ser Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser
      30                  35                  40

caa gcg ctt tca caa cca att gcc tcc tct aac gtt cat gat aac ttc  195
Gln Ala Leu Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe
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Val His Cys Glu Tyr Tyr Leu Leu Ala Ile Ile Tyr Pro His Ser Met
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gtt aaa tct gat att tca att cta gat aat tac cct aaa aaa tgg agg   627
Val Lys Ser Asp Ile Ser Ile Leu Asp Asn Tyr Pro Lys Lys Trp Arg
    190                 195                 200

caa tat tat gat gac gct aat tta ata aaa tat gat cct ata gta gat   675
Gln Tyr Tyr Asp Asp Ala Asn Leu Ile Lys Tyr Asp Pro Ile Val Asp
205                 210                 215                 220

tat tct aac tcc aat cat tca cca att aat tgg aat ata ttt gaa aac   723
Tyr Ser Asn Ser Asn His Ser Pro Ile Asn Trp Asn Ile Phe Glu Asn
                225                 230                 235

aat gct gta aat aaa aaa tct cca aat gta att aaa gaa gcg aaa tca   771
Asn Ala Val Asn Lys Lys Ser Pro Asn Val Ile Lys Glu Ala Lys Ser
            240                 245                 250

tca ggt ctt atc act ggg ttt agt ttc cct att cat act gct aat aat   819
Ser Gly Leu Ile Thr Gly Phe Ser Phe Pro Ile His Thr Ala Asn Asn
        255                 260                 265

ggc ttc gga atg ctt agt ttt gca cat tca gag aaa gac aac tat ata   867
Gly Phe Gly Met Leu Ser Phe Ala His Ser Glu Lys Asp Asn Tyr Ile
    270                 275                 280

gat agt tta ttt tta cat gca tgt atg aac ata cca tta att gtt cct   915
Asp Ser Leu Phe Leu His Ala Cys Met Asn Ile Pro Leu Ile Val Pro
285                 290                 295                 300

tct cta gtt gat aat tat cga aaa ata aat ata gca aat aat aaa tca   963
Ser Leu Val Asp Asn Tyr Arg Lys Ile Asn Ile Ala Asn Asn Lys Ser
                305                 310                 315

aac aac gat tta acc aaa aga gaa aaa gaa tgt tta gcgtgggcat       1009
Asn Asn Asp Leu Thr Lys Arg Glu Lys Glu Cys Leu
            320                 325

gcgaaggaaa gagctcttgg gatatttcaa aaatattagg ctgcagtaag cgtacggtca 1069

ctttccattt aaccaatgcg caaatgaaac tcaatacaac aaaccgctgc caaagtattt 1129

ctaaagcaat tttaacagga gcaattgatt gcccatactt taaatcatga ctcgactgca 1189

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Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu Ser
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Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn Glu
     50                  55                  60
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Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu Ser
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Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr Thr
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Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu Phe
            100                 105                 110

Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser Thr
        115                 120                 125

Pro Gly Ile Gln Ile Leu Thr Met Gly Met Lys Asp Ile Asn Ala Asp
    130                 135                 140

Asp Thr Tyr Arg Ile Ile Asn Lys Ile Lys Ala Cys Arg Ser Asn Asn
145                 150                 155                 160

Asp Ile Asn Gln Cys Leu Ser Asp Met Thr Lys Met Val His Cys Glu
                165                 170                 175

Tyr Tyr Leu Leu Ala Ile Ile Tyr Pro His Ser Met Val Lys Ser Asp
            180                 185                 190

Ile Ser Ile Leu Asp Asn Tyr Pro Lys Lys Trp Arg Gln Tyr Tyr Asp
        195                 200                 205

Asp Ala Asn Leu Ile Lys Tyr Asp Pro Ile Val Asp Tyr Ser Asn Ser
    210                 215                 220

Asn His Ser Pro Ile Asn Trp Asn Ile Phe Glu Asn Asn Ala Val Asn
225                 230                 235                 240

Lys Lys Ser Pro Asn Val Ile Lys Glu Ala Lys Ser Ser Gly Leu Ile
                245                 250                 255

Thr Gly Phe Ser Phe Pro Ile His Thr Ala Asn Asn Gly Phe Gly Met
            260                 265                 270

Leu Ser Phe Ala His Ser Glu Lys Asp Asn Tyr Ile Asp Ser Leu Phe
        275                 280                 285

Leu His Ala Cys Met Asn Ile Pro Leu Ile Val Pro Ser Leu Val Asp
    290                 295                 300

Asn Tyr Arg Lys Ile Asn Ile Ala Asn Asn Lys Ser Asn Asn Asp Leu
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Thr Lys Arg Glu Lys Glu Cys Leu
                325
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<210> 9
<211> 1206
<212> DNA
<213> Artificial Sequence

<220>
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<222> (12)..(1187)

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              1               5                  10

ata att aat aaa att aaa gct tgt aga agc aat aat gat att aat caa   98
Ile Ile Asn Lys Ile Lys Ala Cys Arg Ser Asn Asn Asp Ile Asn Gln
     15                  20                  25

tgc tta tct gat atg act aaa atg gta cat tgt gaa tat tat tta ctc   146
Cys Leu Ser Asp Met Thr Lys Met Val His Cys Glu Tyr Tyr Leu Leu
 30                  35                  40                  45

gcg atc att tat cct cat tcc atg gtt aaa tct gat att tca att cta   194
Ala Ile Ile Tyr Pro His Ser Met Val Lys Ser Asp Ile Ser Ile Leu
                 50                  55                  60

gat aat tac cct aaa aaa tgg agg caa tat tat gat gac gct aat tta   242
Asp Asn Tyr Pro Lys Lys Trp Arg Gln Tyr Tyr Asp Asp Ala Asn Leu
             65                  70                  75

ata aaa tat gat cct ata gta gat tat tct aac tcc aat cat tca cca   290
Ile Lys Tyr Asp Pro Ile Val Asp Tyr Ser Asn Ser Asn His Ser Pro
         80                  85                  90

att aat tgg aat ata ttt gaa aac aat gct gta aat aaa aaa tct cca   338
Ile Asn Trp Asn Ile Phe Glu Asn Asn Ala Val Asn Lys Lys Ser Pro
     95                  100                 105

aat gta att aaa gaa gcg aaa tca tca ggt ctt atc act ggg ttt agt   386
Asn Val Ile Lys Glu Ala Lys Ser Ser Gly Leu Ile Thr Gly Phe Ser
110                 115                 120                 125

ttc cct att cat act gct aat aat ggc ttc gga atg ctt agt ttt gca   434
Phe Pro Ile His Thr Ala Asn Asn Gly Phe Gly Met Leu Ser Phe Ala
                130                 135                 140

cat tca gag aaa gac aac tat ata gat agt tta ttt tta cat gca tgt   482
His Ser Glu Lys Asp Asn Tyr Ile Asp Ser Leu Phe Leu His Ala Cys
            145                 150                 155

atg aac ata cca tta att gtt cct tct cta gtt gat aat tat cga aaa   530
Met Asn Ile Pro Leu Ile Val Pro Ser Leu Val Asp Asn Tyr Arg Lys
        160                 165                 170

ata aat ata gca aat aat aaa tca aac aac gat tta acc aaa aga gaa   578
Ile Asn Ile Ala Asn Asn Lys Ser Asn Asn Asp Leu Thr Lys Arg Glu
    175                 180                 185

aaa gaa tgt tta gcg tgg gca tgc gaa gga aag agc tct tgg gat att   626
Lys Glu Cys Leu Ala Trp Ala Cys Glu Gly Lys Ser Ser Trp Asp Ile
190                 195                 200                 205

tca aaa ata tta ggc tgc agt aag cgt acg gtc act ttc cat tta acc   674
Ser Lys Ile Leu Gly Cys Ser Lys Arg Thr Val Thr Phe His Leu Thr
                210                 215                 220

aat gcg caa atg aaa ctc aat aca aca aac cgc tgc caa agt att tct   722
Asn Ala Gln Met Lys Leu Asn Thr Thr Asn Arg Cys Gln Ser Ile Ser
            225                 230                 235

aaa gca att tta aca gga gca att gat tgc cca tac ttt aaa agt atc   770
Lys Ala Ile Leu Thr Gly Ala Ile Asp Cys Pro Tyr Phe Lys Ser Ile
        240                 245                 250

gat aag ctt atg ccc aag aag aag cgg aag gtc tcg agc ggc gcc aat   818
Asp Lys Leu Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn
    255                 260                 265
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ttt aat caa agt ggg aat att gct gat agc tca ttg tcc ttc act ttc   866
Phe Asn Gln Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe
270                 275                 280                 285

act aac agt agc aac ggt ccg aac ctc ata aca act caa aca aat tct   914
Thr Asn Ser Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser
                290                 295                 300

caa gcg ctt tca caa cca att gcc tcc tct aac gtt cat gat aac ttc   962
Gln Ala Leu Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe
            305                 310                 315

atg aat aat gaa atc acg gct agt aaa att gat gat ggt aat aat tca   1010
Met Asn Asn Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser
        320                 325                 330

aaa cca ctg tca cct ggt tgg acg gac caa act gcg tat aac gcg ttt   1058
Lys Pro Leu Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe
    335                 340                 345

gga atc act aca ggg atg ttt aat acc act aca atg gat gat gta tat   1106
Gly Ile Thr Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr
350                 355                 360                 365

aac tat cta ttc gat gat gaa gat acc cca cca aac cca aaa aaa gag   1154
Asn Tyr Leu Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu
                370                 375                 380

ggt ggg tcg acc ccg gga att cag atc tac tag tgcggccgct cgactgcag  1206
Gly Gly Ser Thr Pro Gly Ile Gln Ile Tyr
            385                 390
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<210> 10
<211> 391
<212> PRT
<213> Artificial Sequence

<400> 10

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Lys Ile Lys Ala Cys Arg Ser Asn Asn Asp Ile Asn Gln Cys Leu Ser
             20                  25                  30

Asp Met Thr Lys Met Val His Cys Glu Tyr Tyr Leu Leu Ala Ile Ile
         35                  40                  45

Tyr Pro His Ser Met Val Lys Ser Asp Ile Ser Ile Leu Asp Asn Tyr
     50                  55                  60

Pro Lys Lys Trp Arg Gln Tyr Tyr Asp Asp Ala Asn Leu Ile Lys Tyr
 65                  70                  75                  80

Asp Pro Ile Val Asp Tyr Ser Asn Ser Asn His Ser Pro Ile Asn Trp
                 85                  90                  95

Asn Ile Phe Glu Asn Asn Ala Val Asn Lys Lys Ser Pro Asn Val Ile
            100                 105                 110

Lys Glu Ala Lys Ser Ser Gly Leu Ile Thr Gly Phe Ser Phe Pro Ile
        115                 120                 125

His Thr Ala Asn Asn Gly Phe Gly Met Leu Ser Phe Ala His Ser Glu
    130                 135                 140
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Lys Asp Asn Tyr Ile Asp Ser Leu Phe Leu His Ala Cys Met Asn Ile
145                 150                 155                 160

Pro Leu Ile Val Pro Ser Leu Val Asp Asn Tyr Arg Lys Ile Asn Ile
                165                 170                 175

Ala Asn Asn Lys Ser Asn Asn Asp Leu Thr Lys Arg Glu Lys Glu Cys
            180                 185                 190

Leu Ala Trp Ala Cys Glu Gly Lys Ser Ser Trp Asp Ile Ser Lys Ile
        195                 200                 205

Leu Gly Cys Ser Lys Arg Thr Val Thr Phe His Leu Thr Asn Ala Gln
    210                 215                 220

Met Lys Leu Asn Thr Thr Asn Arg Cys Gln Ser Ile Ser Lys Ala Ile
225                 230                 235                 240

Leu Thr Gly Ala Ile Asp Cys Pro Tyr Phe Lys Ser Ile Asp Lys Leu
                245                 250                 255

Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln
            260                 265                 270

Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser
        275                 280                 285

Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu
    290                 295                 300

Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn
305                 310                 315                 320

Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu
                325                 330                 335

Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr
            340                 345                 350

Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu
        355                 360                 365

Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser
    370                 375                 380

Thr Pro Gly Ile Gln Ile Tyr
385                 390
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<210> 11
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
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<400> 11
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<210> 12
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:TraR2 Fragment

<400> 12
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<210> 13
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
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Fragment

<400> 13
tcgacacctg cgagttctcc gaggtg 26

<210> 14
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<212> DNA
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<220>
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<400> 14
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<210> 15
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Fragment

<400> 15
tcgactacac gtctagacgt gtagg 25

<210> 16
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<212> DNA
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<220>
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<400> 16
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<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:TraBox3
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<400> 17
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<210> 18
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:TraBox4
Fragment

<400> 18
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<210> 19
<211> 250
<212> PRT
<213> Photobacterium fischeri

<400> 19
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Lys Ala Cys Arg Ser Asn Asn Asp Ile Asn Gln Cys Leu Ser Asp Met
20 25 30

Thr Lys Met Val His Cys Glu Tyr Tyr Leu Leu Ala Ile Ile Tyr Pro
35 40 45

His Ser Met Val Lys Ser Asp Ile Ser Ile Leu Asp Asn Tyr Pro Lys
50 55 60

Lys Trp Arg Gln Tyr Tyr Asp Asp Ala Asn Leu Ile Lys Tyr Asp Pro
65 70 75 80

Ile Val Asp Tyr Ser Asn Ser Asn His Ser Pro Ile Asn Trp Asn Ile
85 90 95

Phe Glu Asn Asn Ala Val Asn Lys Lys Ser Pro Asn Val Ile Lys Glu
100 105 110

Ala Lys Thr Ser Gly Leu Ile Thr Gly Phe Ser Phe Pro Ile His Thr
115 120 125

Ala Asn Asn Gly Phe Gly Met Leu Ser Phe Ala His Ser Glu Lys Asp
130 135 140

Asn Tyr Ile Asp Ser Leu Phe Leu His Ala Cys Met Asn Ile Pro Leu
145 150 155 160

Ile Val Pro Ser Leu Val Asp Asn Tyr Arg Lys Ile Asn Ile Ala Asn
165 170 175

Asn Lys Ser Asn Asn Asp Leu Thr Lys Arg Glu Lys Glu Cys Leu Ala
180 185 190

Trp Ala Cys Glu Gly Lys Ser Ser Trp Asp Ile Ser Lys Ile Leu Gly
195 200 205

Cys Ser Glu Arg Thr Val Thr Phe His Leu Thr Asn Ala Gln Met Lys

210 215 220

Leu Asn Thr Thr Asn Arg Cys Gln Ser Ile Ser Lys Ala Ile Leu Thr
225 230 235 240

Gly Ala Ile Asp Cys Pro Tyr Phe Lys Asn
245 250

<210> 20
<211> 239
<212> PRT
<213> Pseudomonas aeruginosa

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Ser Lys Ile Leu Phe Gly Leu Leu Pro Lys Asp Ser Gln Asp Tyr Glu
35 40 45

Asn Ala Phe Ile Val Gly Asn Tyr Pro Ala Ala Trp Arg Glu His Tyr
50 55 60

Asp Arg Ala Gly Tyr Ala Arg Val Asp Pro Thr Val Ser His Cys Thr
65 70 75 80

Gln Ser Val Leu Pro Ile Phe Trp Glu Pro Ser Ile Tyr Gln Thr Arg
85 90 95

Lys Gln His Glu Phe Phe Glu Glu Ala Ser Ala Ala Gly Leu Val Tyr
100 105 110

Gly Leu Thr Met Pro Leu His Gly Ala Arg Gly Glu Leu Gly Ala Leu
115 120 125

Ser Leu Ser Val Glu Ala Glu Asn Arg Ala Glu Ala Asn Arg Phe Met
130 135 140

Glu Ser Val Leu Pro Thr Leu Trp Met Leu Lys Asp Tyr Ala Leu Gln
145 150 155 160

Ser Gly Ala Gly Leu Ala Phe Glu His Pro Val Ser Lys Pro Val Val
165 170 175

Leu Thr Ser Arg Glu Lys Glu Val Leu Gln Trp Cys Ala Ile Gly Lys
180 185 190

Thr Ser Trp Glu Ile Ser Val Ile Cys Asn Cys Ser Glu Ala Asn Val
195 200 205

Asn Phe His Met Gly Asn Ile Arg Arg Lys Phe Gly Val Thr Ser Arg
210 215 220

Arg Val Ala Ala Ile Met Ala Val Asn Leu Gly Leu Ile Thr Leu
225 230 235

<210> 21
<211> 234
<212> PRT
<213> Agrobacterium tumefaciens

<400> 21
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Asp Glu Cys Ile Leu Lys Asp Gly Leu Ala Asp Leu Ala Glu His Phe
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35 40 45

Ala Val Thr Asn Tyr His Arg Asp Trp Arg Ser Ala Tyr Phe Glu Asn
50 55 60

Asn Phe Asp Lys Leu Asp Pro Val Val Lys Arg Ala Lys Ser Arg Lys
65 70 75 80

His Val Phe Ala Trp Ser Gly Glu Gln Glu Arg Ser Arg Leu Ser Lys
85 90 95

Glu Glu Arg Ala Phe Tyr Ala His Ala Ala Asp Phe Gly Ile Arg Ser
100 105 110

Gly Ile Thr Ile Pro Ile Lys Thr Ala Asn Gly Ser Met Ser Met Phe
115 120 125

Thr Leu Ala Ser Glu Arg Pro Ala Ile Asp Leu Asp Arg Glu Ile Asp
130 135 140

Ala Ala Ala Ala Ala Gly Ala Val Gly Gln Leu His Ala Arg Ile Ser
145 150 155 160

Phe Leu Gln Thr Thr Pro Thr Val Glu Asp Ala Ala Trp Leu Asp Pro
165 170 175

Lys Glu Ala Thr Tyr Leu Arg Trp Ile Ala Val Gly Met Thr Met Glu
180 185 190

Glu Val Ala Asp Val Glu Gly Val Lys Tyr Asn Ser Val Arg Val Lys
195 200 205

Leu Arg Glu Ala Met Lys Arg Phe Asp Val Arg Ser Lys Ala His Leu
210 215 220

Thr Ala Leu Ala Ile Arg Arg Lys Leu Ile
225 230

<210> 22
<211> 720
<212> DNA
<213> Pseudomonas aeruginosa

<400> 22
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cctaaggaca gccaggacta cgagaacgcc ttcatcgtcg gcaactaccc ggccgcctgg 180

cgcgagcatt acgaccgggc tggctacgcg cgggtcgacc cgacggtcag tcactgtacc 240

cagagcgtac tgccgatttt ctgggaaccg tccatctacc agacgcgaaa gcagcacgag 300

ttcttcgagg aagcctcggc cgccggcctg gtgtatgggc tgaccatgcc gctgcatggt 360

gctcgcggcg aactcggcgc gctgagcctc agcgtggaag cggaaaaccg ggccgaggcc 420

aaccgtttca tggagtcggt cctgccgacc ctgtggatgc tcaaggacta cgcactgcag 480

agcggtgccg gactggcctt cgaacatccg gtcagcaaac cggtggttct gaccagccgg 540

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tgcaactgct cggaagccaa tgtgaacttc catatgggaa atattcggcg gaagttcggt 660

gtgacctccc gccgcgtagc ggccattatg gccgttaatt tgggtcttat tactctctga 720

<210> 23
<211> 705
<212> DNA
<213> Agrobacterium tumefaciens

<400> 23
atgcagcact ggctggacaa gttgaccgat cttgccgcaa ttcagggcga cgagtgcatc 60

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catatccagc acaaacacac catcgcggtc accaattatc atcgtgactg gcgatcggct 180

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gccaacggat caatgtcgat gttcacgctg gcgtcggaaa ggccggcgat cgacctcgac 420

cgtgagatcg acgcggccgc agccgcgggc gccgtcgggc agctccatgc ccgcatctct 480

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aaggcccatc tcaccgccct cgcaatcaga agaaagctga tctga 705

<210> 24
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:LuxI Box
Promoter Region

<400> 24
cacctgtagg atcgtacagg t 21

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/GB 99/02653

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/52 C12N15/82 C12Q1/68 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06268 A (ZENECA LTD ;JEPSON IAN (GB); PAINE JACQUELINE ANN MARY (GB)) 20 February 1997 (1997-02-20) see the whole document; pref. p.12-14; examples ---	1,2,8,9, 11-18, 22-24, 28-34
X	WO 96 37609 A (ZENECA LTD ;JEPSON IAN (GB); MARTINEZ ALBERTO (GB); GREENLAND ANDR) 28 November 1996 (1996-11-28) cited in the application see the whole document; pref. p.5-7; examples --- -/--	1,2,8,9, 11-18, 22-24, 28-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1999	22/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

1

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INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/GB 99/02653

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROBSON N D ET AL: "Bacterial N-acyl-homoserine-lactone-dependent signalling and its potential biotechnological applications" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 15, no. 11, page 458-464 XP004092668 ISSN: 0167-7799 see esp. p.462 l. par. - p.464; figure 3 ---	1-34
A	US 5 196 318 A (BALDWIN THOMAS O ET AL) 23 March 1993 (1993-03-23) the whole document ---	1-34
A	US 5 759 798 A (DUNLAP PAUL VERNON) 2 June 1998 (1998-06-02) the whole document -----	32

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ial Application No
PCT/GB 99/02653

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9706268 A	20-02-1997	AU 704172 B	15-04-1999
		AU 6625296 A	05-03-1997
		BR 9609873 A	23-03-1999
		CN 1199425 A	18-11-1998
		CZ 9800369 A	17-06-1998
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